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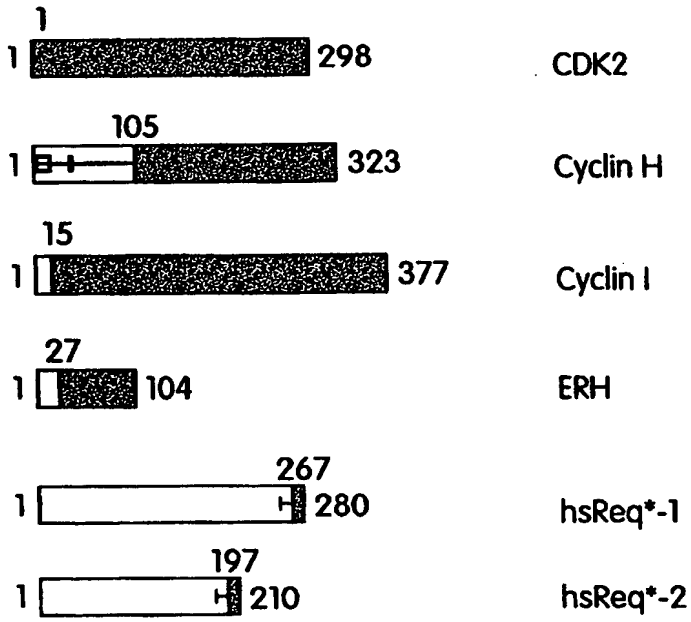
(54) Title: CDK2 PROTEIN AND CDK2 PROTEIN COMPLEXES

(57) Abstract

The present invention discloses complexes of the CDK2 protein with proteins identified as interacting with the CDK2 protein (CDK2 protein–IPs) by a modified, improved yeast two hybrid assay system. The proteins which were identified to interact with the CDK2 protein, and thus form complexes, included: cyclin I, ERH, hsReq*–1 and hsReq*–2, as well as derivatives, fragments analogs and homologs thereof. The invention also provides nucleic acids encoding the hsReq*–1 and hsReq*–2 nucleotide sequences, and proteins and derivatives, fragments and analogs thereof. Methodologies of screening these aforementioned complexes for efficacy in treating and/or preventing various diseases and disorders, particularly neoplasia and atherosclerosis, are also disclosed herein.



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CDK2 PROTEIN AND CDK2 PROTEIN COMPLEXES

GRANT SUPPORT

This invention was made with United States Government support under award number 70NANB5H1066 awarded by the National Institute of Standards and Technology. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention disclosed herein relates to complexes of the CDK2 protein with other proteins, in particular, complexes of the CDK2 protein with the following proteins: cyclin I, ERH, hsReq*-1 and hsReq*-2. In addition, the present invention relates to the production of antibodies to the aforementioned CDK2 protein complexes, and their use in, *inter alia*, screening, diagnosis, prognosis and therapy. The present invention further relates to the hsReq*-1 and hsReq*-2 genes and proteins, as well as derivatives, fragments, analogs and homologs, thereof.

BACKGROUND OF THE INVENTION

It is a well-established tenet in molecular biology that loss of control of cell proliferation may lead to severe diseases and disorders (*e.g.*, neoplasia). Hence, the elucidation of the intricacies of the cell-cycle, and its deregulation during oncogenesis, will provide novel opportunities in the prophylactic, diagnostic and therapeutic management of cancer and other proliferation-related diseases. A better understanding of the cell-cycle could be achieved by the elucidation of the interactions of the various protein complexes, whose levels and biological activities are regulated through the cell-cycle. The identification and classification of these protein complexes will be useful in the development of treatment modalities and assays for

various pathological processes including, but not limited to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression), as well as atherosclerosis.

It should be noted that the citation of a reference herein should not be construed as an admission that such is prior art to the present invention.

(1) The CDK2 Protein

Human cyclin-dependent kinase 2 or cell division kinase (CDK2; GenBank Accession No. X61622; see Elledge & Spottswood, 1991. *EMBO J.* 10:2653-2659; Ninomiya-Tsuji, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:9006-9010) is a serine-threonine protein kinase of 298 amino acids that has approximately 65% amino acid identity to a second critical cell cycle regulator, CDC2. CDK2 is expressed late in G1 or early in S phase slightly before CDC2, and is pivotal for G1/S transition. The two kinases regulate the cell cycle at distinct stages.

Cyclin-dependent kinases (CDKs) form complexes with cyclins, and as a consequence they generally express kinase activities. One of these CDKs, CDK2, is known to bind with cyclins (*e.g.*, with cyclins A, E, D1 and H), and plays an important role in the progression of the cell cycle via phosphorylation of target proteins. CDK2 activity is dependent upon phosphorylation by CDK-activating kinase that occurs when CDK2 complexes with the cell cycle regulators cyclins A and E. Conversely, CDK2 kinase activity is inactivated by dephosphorylation by human CDK-associated phosphatase (see *e.g.*, Poon & Hunter, 1995. *Science* 270:90-93). CDK2 phosphorylates the retinoblastoma tumor-suppressor gene product (pRb), p53, transcription factor E2F, histone H1, and other proteins central to cell cycle control (see *e.g.*, Higashi, *et al.*, 1996. *Eur. J. Biochem.* 237:460-467). Other proteins, including cyclin D1 and p21, complex with CDK2 to block its interaction with downstream substrates, as well as blocking CDK2 phosphorylation itself (see *e.g.*, Adams, *et al.*, 1996. *Mol. Cell. Biol.* 16:6623-6633). The complex interplay of phase-specific cyclin expression, phosphorylation/ dephosphorylation cascades, and other CDK2 interacting proteins ultimately plays out through CDK2 activity to determine cell cycle progression.

Deregulation of CDK2 is strongly implicated in mechanisms of carcinogenesis and in the treatment of cancer. DNA tumor viruses transform cells through CDK2 interaction with transcription factor E2F (see *e.g.*, Nevins, 1992. *Science* 258:424-429). CDK2 is implicated in the differentiation of glioma cells (see *e.g.*, Kokunai, *et al.*, 1997. *J. Neuro. Oncol.* 32:125-133).

In human breast carcinoma cells, the anti-cancer agent flavopiridol induces G1 arrest by inhibition of CDK2 (see *e.g.*, Carlson, *et al.*, 1996. *Cancer Res.* 56:2973-2978). Anti-estrogens up-regulate CDK2 inhibitors, thus causing reduction in pRb phosphorylation, and decreased cell progression into S phase (see *e.g.*, Watts, *et al.*, 1995. *Mol. Endocrinol.* 9:1804-1813).

Smooth muscle cell proliferation is a key event in the development of atherosclerosis. Serum-deprivation of vascular smooth muscle cells is associated with a complex formation between CDK2 and p27(Kip1), leading to inhibition of CDK2 enzymatic activity (see *e.g.*, Chen, *et al.*, 1997. *J. Clin. Invest.* 99:2334-2341). Thus, inhibiting CDK2/cyclin E activity in the G1 phase of the cell cycle is the mechanism through which p27(Kip1) acts to inhibit intimal hyperplasia during atherosclerosis.

To review, CDK2 is implicated in the control of cell cycle progression, transcriptional regulation, control of cellular differentiation, intracellular signal transduction involving phosphorylation, mechanisms of tumorigenesis, tumor progression and spread, and atherosclerosis.

(2) CDK2 interacting proteins

(*i*) Cyclin I

Cyclin I (GenBank Accession No. D50310; see Nakamura, *et al.*, 1995. *Exp. Cell Res.* 221:534-542), in contrast to other cyclin proteins, is widely expressed in many post-mitotic tissues at constant levels throughout the cell cycle. The protein contains a typical cyclin box near the amino-terminus, implicating it in control of cell cycle progression and transcriptional control (see *e.g.*, Gibson, *et al.*, 1994. *Nucleic Acids Res.* 22:946-952).

(ii) ERH

A human cDNA (GenBank Accession No. D85785; see Isomura, *et al.*, 1996) encoding a 104 amino acid protein termed ERH (for human enhancer of rudimentary gene), homologous to the enhancer of the rudimentary gene in *Drosophila melanogaster* (DROER), was found to interact with CDK2 in the present invention. In *Drosophila*, the gene product is required for transcriptional regulation of the rudimentary gene in *Drosophila melanogaster*. The protein has been implicated in the pyrimidine metabolic pathway, and the cell cycle. ERH is thus implicated to function in transcriptional control, DNA pyrimidine metabolism, and in the cell cycle.

(iii) hsReq*-1 and hsReq*-2

Two sequences were identified in this invention as CDK2 interactants that are identical to sequences within the human homologue of the mouse zinc finger protein Requiem (hsReq; GenBank Accession No. U94585; see Gabig, *et al.*, 1994. *J. Biol. Chem.* 269:29515-29519). Apoptosis in murine myeloid cell lines requires the expression of the Requiem gene. The hsReq regions identified in this invention represent a splice variant of hsReq containing amino acids encoded by a nucleotide sequence of the 3' untranslated region of the hsReq mRNA. Two such splice variants are disclosed *infra*, and are designated as hsReq*-1 and hsReq*-2 in the present invention.

It should be noted that there has been no previous disclosure within the prior art of any type of interaction of CDK2, cyclin I, ERH, hsReq*-1 and hsReq*-2, as described *infra*.

Additionally, citation of a reference herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY OF THE INVENTION

In brief, the CDK2 protein has been demonstrated to form complexes, which heretofore have not been described, with the following cellular proteins: cyclin I, ERH, hsReq*-1 and hsReq*-2. In addition, the genes which encode the hsReq*-1 and hsReq*-2 proteins have not been previously described.

The present invention discloses herein compositions and methodologies for the production of protein complexes comprised of the CDK2 protein and various other proteins which interact with (*i.e.*, bind to) said CDK2 protein. The proteins which have been demonstrated to form complexes with the CDK2 protein will be designated hereinafter as "CDK2 protein-IP" for CDK2 protein interacting protein; whereas a complex of the CDK2 protein and a CDK2 protein-IP will hereinafter be designated as "CDK2 protein•CDK2 protein-IP".

More specifically, the present invention relates to complexes of the CDK2 protein, and derivatives, fragments and analogs thereof, with the following cellular proteins: (*i*) cyclin I; (*ii*) ERH, (*ii*) hsReq*-1; (iv) hsReq*-2, as well as their derivatives, analogs and fragments. hsReq*-1 and hsReq*-2 are novel proteins encoded by mRNA splice variants of the hsReq gene, i.e. the mRNAs encoding hsReq*-1 and hsReq*-2 are generated by RNA splicing at splice sites other than the splice sites used to process the mRNA encoding hsReq. Accordingly, the invention further relates to nucleotide sequences of hsReq*-1 and hsReq*-2 (human hsReq*-1 and hsReq*-2 genes and homologs of other species), as well as derivatives (*e.g.*, fragments) and analogs thereof.

Methods of production of the CDK2 protein•CDK2 protein-IP complexes, and derivatives and analogs of these aforementioned proteins and protein complexes by, for example, recombinant means, will also be disclosed herein. Various pharmaceutical compositions relating to the CDK2 protein:CDK2 protein-IPs, CDK2 protein•CDK2 protein-IP complexes, and derivatives, fragments and analog thereof, will also be disclosed by the present invention.

The present invention will further provide methodologies for the modulation (*i.e.*, inhibiting or enhancing) of the activity of the CDK2 protein•CDK2 protein-IP complexes, particularly: the following complexes: CDK2 protein•cyclin I; CDK2 protein•ERH, CDK2 protein•hsReq*-1 and CDK2 protein•hsReq*-2. The protein components of these aforementioned complexes have been implicated in a plethora of cellular and physiological processes, including, but not limited to: (*i*) control of cell-cycle progression; (*ii*) cellular differentiation and apoptosis; (*iii*) regulation of transcription; (iv) pathological processes including, but not restricted to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression); and atherosclerosis.

Accordingly, the present invention provides methodologies for the screening of CDK2 protein•CDK2 protein-IP complexes, particularly complexes of the CDK2 protein with cyclin I, ERH, hsReq*-1 and hsReq*-2, as well as derivatives, fragments and analogs thereof, for the ability to modulate or alter cell functions, particularly those cell functions in which CDK2 protein and/or a CDK2 protein-IP has been implicated including, but not limited to: (*i*) control of cell-cycle progression; (*ii*) cellular differentiation and apoptosis; (*iii*) regulation of transcription; (iv) pathological processes including, but not restricted to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression); and atherosclerosis.

The present invention further relates to therapeutic and prophylactic, as well as diagnostic, prognostic and screening methodologies and pharmaceutical compositions which are based upon CDK2 protein•CDK2 protein-IP complexes (and the nucleic acids encoding the individual proteins constituents which participate in the complexes). Therapeutic compounds of the invention include, but are not limited to: (*i*) CDK2 protein•CDK2 protein-IP complexes, and complexes where one or both members of the complex is a derivative, fragment or analog of the CDK2 protein or a CDK2 protein-IP; (*ii*) antibodies to, and nucleic acids encoding the foregoing and (*iii*) antisense nucleic acids to the nucleotide sequences encoding the various protein complex components. Diagnostic, prognostic and screening kits will also be provided.

Animal models and methodologies of screening for various modulatory agents (*i.e.*, agonists, antagonists and inhibitors) of the activity of the CDK2 protein:CDK2 protein-IPs and CDK2 protein•CDK2 protein-IP complexes, are also disclosed herein.

Methodologies for the identification of molecules which inhibit, or alternatively, which increase the formation/synthesis of the CDK2 protein:CDK2 protein-IPs and CDK2 protein• CDK2 protein-IP complexes will also be provided by the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the present invention disclosed herein is better understood and appreciated, the following detailed description is set forth.

Figure 1: The nucleotide sequence of CDK2 (GenBank Accession No. X61622 (SEQ ID NO: 1)) and deduced amino acid sequence (SEQ ID NO:2). The coding sequence in its entirety was used as bait in the assays described in Section 6, *infra*.

Figure 2: The nucleotide sequence (SEQ ID NO:3) and corresponding amino acid sequence (SEQ ID NO:4) of the cyclin I protein (GenBank Accession No. D50310). The prey sequence identified in the assay described in Section 6, *infra*, begins at base 46 (amino acid 16) and is indicated by arrow "A".

Figure 3: The nucleotide acid sequence (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6) of ERH (GenBank Accession No. D85758). The prey sequence identified in the assay described in Section 6, *infra*, begins at base 153 (amino acid 27) and is indicated by arrow "A".

Figure 4: The nucleotide sequence of *hsReq* (GenBank Accession No. U94585; SEQ ID NO:7). The prey sequence identified in Section 6 *infra* and beginning at base 1789, is underlined. The second prey sequence identified in Section 6, *infra* and beginning at base 1819, is over-lined. The initiation methionine codon ATG of hsReq is marked as "A", and the stop codon TGA for hsReq is marked as "C". A 5' splice site, with bases identical to the known consensus sequence for 5' splice sites is shown in bold, and the last base of the exon (exon 1) is marked by arrow "B". A 3' splice site, with bases identical to the known consensus sequence for 3' splice sites is shown in bold, and the first \base of the exon (exon 2) is marked by arrow "E", and the stop codon TGA for *hsReq*-1* is marked by "H". The branch point consensus sequence for this exon, with bases matching the consensus bases shown in bold, is marked as "D". An alternate 3' splice site is marked as "G", with the associated branch splice point marked as "F". The stop codon TGA in this exon for *hsReq*-2*, is indicated as "H". The AAUAAA transcriptional stop signal near the end of the sequence is marked as "I".

Figure 5: The hsReq*-1 nucleotide acid sequence (SEQ ID NO:8) and amino acid sequence (SEQ ID NO:9). The amino-terminal amino acid residue of the amino acid sequence that differs from hsReq because of alternate splicing is marked by arrow "A". One prey sequence identified in the assay described in Section 6, *infra*, begins at base 1789 of the *hsReq* sequence (Figure 4), and is indicated by arrow "B". The second prey sequence identified in the assay described in Section 6, *infra*, begins at base 1819 of the *hsReq* sequence (Figure 4) and is indicated by arrow "C".

Figure 6: The hsReq*-2 nucleotide acid sequence (SEQ ID NO:10) and amino acid sequence (SEQ ID NO:11). The amino acid sequence carboxyl-terminal to the amino acid marked by the arrow "A" deviates from the amino acid sequence of hsReq because of alternate splicing. One prey sequence identified in the assay described in Section 6, *infra*, begins at base 1789 of the *hsReq* sequence (Figure 4), and is indicated by arrow "A". The second prey

sequence identified in the assay described in Section 6, *infra*, begins at base 1819 of the *hsReq* sequence (Figure 4), and is indicated by arrow "B".

Figure 7: Schematic of the portions of CDK2, cyclin I, ERH, hsReq*-1 and hsReq*-2 that form a CDK2:CDK2-IP complex in the modified yeast two hybrid assay system. The amino acid sequences of CDK2, cyclin I, ERH, hsReq*-1 and hsReq*-2 proteins are depicted as bars, with the starting and ending amino acid numbers indicated above the bars (as depicted for each protein in Figures 1-3 and 5-6 (SEQ ID NOS: 2, 4, 6, 11 and 13, respectively)). The portions of CDK2 used as bait, or the shortest sequences identified as interacting in the assay ("prey sequence") in the case of cyclin I, ERH, hsReq*-1 and hsReq*-2, are blackened and the first amino acid number of that prey sequence is indicated above each bar. In cases where more than one independent prey isolate was identified, *i.e.*, for hsReq*-1, and hsReq*-2, the start sites for the longer prey sequences are indicated by bars, drawn to scale, that extend towards the amino terminus.

Figure 8: Matrix of results of the modified yeast two hybrid system assays. The results of assays using the bait proteins B1 and CDK2 are indicated to the left of the rows, and the prey proteins cyclin H (Cyc. H), ERH, $p27^{kip}$, P1, $p21^{waf}$, and hsReq are indicated above the columns. A positive interaction for a bait and prey protein is indicated as "+" in the box forming the intersection between the particular bait and prey proteins; a lack of interaction is designated by an empty box. Boxes labeled A, B, C, D and E indicate the results of matings and growth of yeast expressing CDK2 and Cyclin H (Cyc. H), ERH, $p27^{kip}$, $p21^{waf}$, and hsReq, respectively. The box labeled F indicates the mating and growth of yeast expressing B1 and P1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the identification of proteins which have been demonstrated to interact with the CDK2 protein (hereinafter referred to as "CDK2 protein-IPs") using an improved, modified form of the yeast two hybrid system. The following proteins (CDK2 protein-IPs) were found to form complexes under physiological conditions with the CDK2 protein: (*i*) cyclin I; (*ii*) ERH; (*iii*) hsReq*-1; (*iv*) hsReq*-2. Complexes of the CDK2 protein with a CDK2 protein-IP are hereinafter referred to as "CDK2 protein•CDK2 protein-IP" complexes. CDK2 protein•CDK2 protein-IP complexes are implicated in the modulation of functional activities of the CDK2 protein and its binding partners (CDK2 protein-IPs). Such functional activities include, but are not limited to: (*i*) control of cell-cycle progression; (*ii*) cellular differentiation and apoptosis; (*iii*) regulation of transcription: (iv) pathological processes including, but not restricted to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression); and atherosclerosis.

The present invention, through utilization of an improved, modified form of the yeast two hybrid system, has identified novel proteins, encoded by the *hsReq*-1 and hsReq*-2* nucleotide sequences. Accordingly, the invention further relates to nucleotide sequences *hsReq*-1 and hsReq*-2* (preferably, the human *hsReq*-1 and hsReq*-2* genes) and homologs of other species, as well as derivatives, fragments and analogs thereof. Nucleic acids which are able to hybridize to, or are complementary to, the aforementioned nucleotide sequence (*e.g.*, the inverse complement) of the foregoing sequences are also provided. More specifically, the present invention discloses nucleic acids which comprise, are hybridizable (*e.g.*, the inverse complement) or which are complementary to, at least a 5, 10 or 25 nucleotide region of the *hsReq*-1 and hsReq*-2* nucleotide sequences.

The present invention also relates to hsReq*-1 and hsReq*-2 derivatives, fragments and analogs which are functionally active (*i.e.*, they are capable of displaying one or more known functional activities of a wild-type hsReq*-1 and hsReq*-2 protein). Such functional activities include, but are not limited to: (*i*) the ability to bind with, or compete for binding with the CDK2 protein; (*ii*) antigenicity (the ability to bind, or compete with, hsReq*-1 and hsReq*-2 for binding to an anti-hsReq*-1 and anti- hsReq*-2 antibody, respectively) and (*iii*) immunogenicity (the ability to generate an antibody which binds hsReq*-1 and hsReq*-2, respectively).

The present invention further discloses methodologies of screening for proteins which interact with (*e.g.*, bind to) the CDK2 protein. The invention also relates to CDK2 protein complexes, in particular the CDK2 protein complexed with one of the following proteins: cyclin I, ERH, hsReq*-1 and hsReq*-2. The invention further discloses complexes of the CDK2 protein, or derivatives, analogs and fragments of the CDK2 protein with cyclin I, ERH, hsReq*-1 and hsReq*-2, or derivatives, analogs and fragments thereof. In a preferred embodiment, such complexes bind an anti-CDK2 protein•CDK2 protein-IP complex antibody. In another specific embodiment, complexes of human CDK2 protein with human proteins are disclosed.

The present invention also provides methodologies for the production and/or isolation of CDK2 protein•CDK2 protein-IP complexes. In a specific embodiment, the present invention provides methodologies of using recombinant DNA techniques to express both the CDK2 protein and its binding partner (CDK2 protein-IP), or fragments, derivatives or homologs of one or both members of the complex; wherein either both binding partners are under the control of one heterologous promoter (*i.e.* a promoter which is not naturally associated with the native gene encoding the particular complex component) or where each is under the control of a separate heterologous promoter.

Methodologies of diagnosis, prognosis, and screening for diseases and disorders associated with aberrant levels of CDK2 protein•CDK2 protein-IP complexes are discloses. The present invention also provides methodologies for the treatment and prevention of diseases or disorders which are associated with aberrant levels of CDK2 protein•CDK2 protein-IP complexes, or aberrant levels or activity of one or more of the components of a CDK2 protein• CDK2 protein-IP complex, by the administration of CDK2 protein•CDK2 protein-IP complexes, or modulators of CDK2 protein•CDK2 protein-IP complex formation or activity (*e.g.*, antibodies which bind the CDK2 protein•CDK2 protein-IP complex, or non-complexed CDK2 protein, or its binding partner (CDK2 protein-IP), or a fragment thereof. Preferably, the aforementioned fragment contains: (*i*) the portion of the CDK2 protein or the CDK2 protein-IP which is directly involved in complex formation; (*ii*) mutants of the CDK2 protein or the CDK2 protein-IP which increase or decrease binding affinity; (*iii*) small molecule inhibitors/enhancers of complex formation; (*iv*) antibodies that either stabilize or neutralize the complex, and the like.

Methodologies of assaying CDK2 protein•CDK2 protein-IP complexes for biological activity as a therapeutic or diagnostic, as well as methods of screening for CDK2 protein•CDK2

protein-IP complex, or modulators thereof (*i.e.*, inhibitors, agonists and antagonists) are also disclosed herein.

For clarity of disclosure and enablement, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

(1) The CDK2 Protein, CDK2 Protein-IPs and CDK2 Protein•CDK2 Protein-IP Complexes

The present invention discloses CDK2 protein•CDK2 protein-IP complexes and, in particular aspects, complexes of the CDK2 protein with: cyclin I, ERH, hsReq*-1 and hsReq*-2. In a preferred embodiment, the CDK2 protein•CDK2 protein-IP complexes are complexes of human proteins. The present invention also relates to: (*i*) complexes of derivatives, fragments and analogs of the CDK2 protein with a CDK2 protein-IP; (*ii*) complexes of the CDK2 protein with derivatives, fragments and analogs of a CDK2 protein-IP and (*iii*) complexes of derivatives, fragments and analogs of the CDK2 protein and a CDK2 protein-IP. It should be noted that, as used herein, fragment, derivative or analog of a CDK2 protein•CDK2 protein-IP complex includes complexes where one or both members of the complex are fragments, derivatives or analogs of the wild-type CDK2 protein or CDK2 protein-IP.

Preferably, as disclosed by the present invention, the CDK2 protein•CDK2 protein-IP complexes in which one or both members of the complex are a fragment, derivative or analog of the wild-type protein are functionally active CDK2 protein•CDK2 protein-IP complexes. In particular aspects, the native proteins, derivatives or analogs of the CDK2 protein and/or the CDK2 protein-IPs are of animals (*e.g.*, mouse, rat, pig, cow, dog, monkey, frog); insects (*e.g.*, fly); plants or, most preferably, human. As utilized herein, the term "functionally active CDK2 protein•CDK2 protein-IP complex" refers to species displaying one or more known functional attributes of a full-length CDK2 protein complexed with a full-length CDK2 protein-IP (*e.g.*, cyclin I, ERH, hsReq*-1 and hsReq*-2) including, but not exclusive to, the control of cellular and physiological processes, such including, but not limited to: (*i*) control of cell-cycle progression; (*ii*) cellular differentiation and apoptosis; (*iii*) regulation of transcription; (*iv*) pathological processes including, but not restricted to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression); and atherosclerosis.

In accord, the present invention provides methodologies for the screening of CDK2 protein•CDK2 protein-IP complexes, particularly complexes of the CDK2 protein with: cyclin I,

ERH, hsReq*-1 and hsReq*-2, as well as derivatives, fragments and analogs thereof, for the ability to alter and/or modulate cellular functions, particularly those functions in which the CDK2 protein and/or CDK2 protein-IP have been implicated. These functions include, but are not limited to: control of cell-cycle progression; regulation of transcription; control of intracellular signal transduction; and pathological processes, as well as various other biological activities (*e.g.*, binding to an anti-CDK2 protein•CDK2 protein-IP complex antibody, and the like). The derivatives, fragments or analogs which possess the desired immunogenicity and/or antigenicity may be utilized in immunoassays, for immunization, for inhibition of CDK2 protein •CDK2 protein-IP complex activity, etc. For example, derivatives, fragments or analogs that retain, or alternatively lack or inhibit, a given property of interest (*e.g.*, participation in a CDK2 protein•CDK2 protein-IP complex) may be utilized as inducers, or inhibitors, respectively, of such a property and its physiological correlates. In a specific embodiment, a CDK2 protein• CDK2 protein-IP complex of a fragment of the CDK2 protein and/or a fragment of CDK2 protein-IP which can be bound by an anti-CDK2 protein and/or anti-CDK2 protein-IP antibody or antibody specific for a CDK2 protein•CDK2 protein-IP complex when such a fragment is included within a given CDK2 protein•CDK2 protein-IP complex. Derivatives, fragments and analogs of CDK2 protein•CDK2 protein-IP complexes may be analyzed for the desired activity or activities by procedures known within the art.

Specific embodiments of the present invention disclose CDK2 protein•CDK2 protein-IP complexes comprised of fragments of one or both protein species of the complex. In a preferred embodiment, these aforementioned fragments may consist of, but are not limited to, fragments of: cyclin I, ERH, hsReq*-1 and hsReq*-2, which have been identified as interacting with the CDK2 protein in an improved, modified yeast two hybrid assay in this invention. For example, amino acids 16-377 of cyclin I protein (depicted in Figure 2; SEQ ID NO:4); amino acid 27-104 of ERB protein (depicted in Figure 3; SEQ ID NO:6); at least amino acid residue 258-, and 267-280 of the hsReq*-1 protein (depicted in Figure; SEQ ID NO:9); at least amino acid residues 188-, and 197-210 of the hsReq*-2 protein (depicted in Figure; SEQ ID NO:11). In addition, fragments (or proteins comprising fragments) which may lack some or all of the aforementioned regions of either member of the complex, as well as nucleic acids which encode the aforementioned proteins, are also disclosed herein.

The present invention further relates to the hsReq*-1 and hsReq*-2 proteins, as well as derivatives, fragments, analogs, homologs and paralogs thereof. In a preferred embodiment, human *hsReq*-1 and hsReq*-2* genes and/or proteins are disclosed. In a specific embodiments, the derivative, fragment, analog, homolog or paralog has the following attributes: (*i*) is functionally active (*i.e.*, capable of exhibiting one or more functional activities associated with full-length, wild-type hsReq*-1 and hsReq*-2; (*ii*) possesses the ability to bind the CDK2 protein; (*iii*) is immunogenic or (*iv*) is antigenic.

The nucleotide sequences which encode, as well as the corresponding amino acid sequences of, human CDK2 protein, cyclin I, and ERB are known (GenBank Accession Nos. X61622, U11791, D50310, and D85758)., respectively), are provided in Figures 1-4, respectively and are identified by SEQ ID NOS:1-8, respectively. In addition, the nucleotide and inferred amino acid sequences hsReq*-1 and hsReq*-2 are provided in Figures 6 and 7, respectively (SEQ ID NOS: 8-12, respectively). Nucleic acids may be obtained by any method known within the art (*e.g.*, by PCR amplification using synthetic primers hybridizable to the 3'- and 5'-termini of the sequence and/or by cloning from a cDNA or genomic library using an oligonucleotide sequence specific for the given gene sequence.

Homologs (*i.e.*, nucleic acids encoding the aforementioned proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can also be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

The CDK2 protein, cyclin I, ERH, hsReq*-1 and hsReq*-2 proteins, either alone or within a complex, may be obtained by methods well-known in the art for protein purification and recombinant protein expression. For recombinant expression of one or more of the proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein may be inserted into an appropriate expression vector (*i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein coding sequence). In a preferred embodiment, the regulatory elements are heterologous (*i.e.*, not the native gene promoter). Alternately, the necessary transcriptional and translational signals may also be supplied by the native promoter for the CDK2 protein or any CDK2 protein-IP genes and/or their flanking regions.

A variety of host-vector systems may be utilized to express the protein coding sequence(s). These include, but are not limited to: (*i*) mammalian cell systems which are infected with vaccinia virus, adenovirus, and the like; (*ii*) insect cell systems infected with baculovirus and the like; (*iii*) yeast containing yeast vectors or (*iv*) bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

In a preferred embodiment of the present invention, the CDK2 protein•CDK2 protein-IP complexes are obtained by expressing the entire CDK2 protein coding sequence and a CDK2 protein-IP coding sequence within the same cell, either under the control of the same promoter or two separate promoters. In another embodiment, a derivative, fragment or homolog of the CDK2 protein and/or a derivative, fragment or homolog of a CDK2 protein-IP are recombinantly expressed. Preferably, the derivative, fragment or homolog of the CDK2 protein and/or the CDK2 protein-IP form a complex with a binding partner which has been identified by a binding assay (*e.g.*, the modified yeast two hybrid system assay) and, more preferably, form a complex which binds to an anti-CDK2 protein•CDK2 protein-IP complex antibody.

Any of the methodologies known within the relevant prior art regarding the insertion of nucleic acid fragments into a vector may be utilized to construct expression vectors which contain a chimeric gene comprised of the appropriate transcriptional/translational control signals and protein-coding sequences. These methodologies may include, but are not limited to, *in vitro* recombinant DNA and synthetic techniques, as well as *in vivo* recombination techniques (*e.g.*, genetic recombination). The expression of nucleic acid sequences which encode the CDK2 protein and a CDK2 protein-IP, or derivatives, fragments, analogs or homologs thereof, may be regulated by a second nucleic acid sequence such that the genes or fragments thereof are expressed in a host which has been concomitantly transformed with the recombinant DNA molecule(s) of interest. The expression of the specific proteins may be controlled by any promoter/enhancer known in the art including, but not limited to: (*i*) the SV40 early promoter (see *e.g.*, Bernoist & Chambon, 1981. *Nature* 290:304-310); (*ii*) the promoter contained within the 3'-terminus long terminal repeat of Rous Sarcoma Virus (RSV; see *e.g.*, Yamamoto, *et al.*, 1980. *Cell* 22:787-797); (*iii*) the Herpesvirus thymidine kinase promoter (see *e.g.*, Wagner, *et al.*, 1981. *Proc. Natl. Acad. Sci. USA* 78:1441-1445); (*iv*) the regulatory sequences of the metallothionein gene (see *e.g.*, Brinster, *et al.*, 1982. *Nature* 296:39-42); (*v*) prokaryotic

expression vectors such as the β-lactamase promoter (see *e.g.*, Villa-Kamaroff, *et al.*, 1978. *Proc. Natl. Acad. Sci. USA* 75:3727-3731); (*vi*) the *tac* promoter (see *e.g.*, DeBoer, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:21-25.

In addition, plant promoter/enhancer sequences within plant expression vectors may also be utilized including, but not limited to: (*i*) the nopaline synthetase promoter (see *e.g.*, Herrar-Estrella, *et al.*, 1984. *Nature* 303:209-213); (*ii*) the cauliflower mosaic virus 35S RNA promoter (see *e.g.*, Garder, *et al.*, 1981. *Nuc. Acids Res.* 9:2871) and (*iii*) the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (see *e.g.*, Herrera-Estrella, *et al.*, 1984. *Nature* 310:115-120).

Promoter/enhancer elements from yeast and other fungi (*e.g.*, the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter), as well as the following animal transcriptional control regions, which possess tissue specificity and have been used in transgenic animals, may be utilized in the production of proteins of the present invention. Transcriptional control sequences derived from animals include, but are not limited to: (*i*) the elastase I gene control region active within pancreatic acinar cells (see *e.g.*, Swift, *et al.*, 1984. *Cell* 38:639-646; Ornitz, *et al.*, 1986. *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409); (*ii*) the insulin gene control region active within pancreatic β-cells (see *e.g.*, Hanahan, *et al.*, 1985. *Nature* 315:115-122); (*iii*) the immunoglobulin gene control region active within lymphoid cells (see *e.g.*, Grosschedl, *et al.*, 1984. *Cell* 38:647-658); (*iv*) the mouse mammary tumor virus control region active within testicular, breast, lymphoid and mast cells (see *e.g.*, Leder, *et al.*, 1986. *Cell* 45:485-495); (*v*) the albumin gene control region active within liver (see *e.g.*, Pinckert, *et al.*, 1987. *Genes and Devel.* 1:268-276); (*vi*) the α-fetoprotein gene control region active within liver (see *e.g.*, Krumlauf, *et al.*, 1985. *Mol. Cell. Biol.* 5:1639-1648; (Hammer *et al.*, 1987, Science 235: 53-58), (*vii*) the α-1 anti-trypsin gene control region active within liver (see *e.g.*, Kelsey, *et al.*, 1987. *Genes and Devel.* 1:161-171); (*viii*) the β-globin gene control region active within myeloid cells (see *e.g.*, Mogram, *et al.*, 1985. *Nature* 315:338-340; (*ix*) the myelin basic protein gene control region active within brain oligodendrocyte cells (see *e.g.*, Readhead, *et al.*, 1987. *Cell* 48:703-712); (*x*) the myosin light chain-2 gene control region active within skeletal muscle (see *e.g.*, Sani, *et al.*, 1985. *Nature* 314:283-286) and (*xi*) the gonadotrophin-releasing hormone gene control region active within the hypothalamus (see *e.g.*, Mason, *et al.*, 1986. *Science* 234:1372-1378).

In a specific embodiment of the present invention, a vector is utilized which comprises a promoter operably-linked to nucleic acid sequences which encode the CDK2 protein and/or a CDK2 protein-IP (*e.g.*, cyclin I, ERH, hsReq*-1 and hsReq*-2), or a fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). In a preferred embodiment, a vector is utilized which is comprised of a promoter operably-linked to nucleic acid sequences encoding both the CDK2 protein and a CDK2 protein-IP, one or more origins of replication, and, optionally, one or more selectable markers.

In another specific embodiment, an expression vector containing the coding sequences (or portions thereof) of the CDK2 protein and a CDK2 protein-IP, either together or separately. The expression vector is generated by subcloning the aforementioned gene sequences into the EcoRI restriction site of each of the three available pGEX vectors (glutathione-S-transferase expression vectors; see *e.g.*, Smith & Johnson, 1988. *Gene* 7:31-40), thus allowing the expression of products in the correct reading frame. Expression vectors which contain the sequences of interest may be identified by three general approaches: (*i*) nucleic acid hybridization, (*ii*) presence or absence of "marker" gene function and/or (*iii*) expression of the inserted sequences. In the first approach, CDK2 protein, cyclin I, ERH, hsReq*-1 and hsReq*-2 (or other CDK2 protein-IP sequences) may be detected by nucleic acid hybridization using probes comprising sequences homologous and complementary to the inserted sequences of interest. In the second approach, the recombinant vector/host system may be identified and selected based upon the presence or absence of certain "marker" functions (*e.g.*, binding to an antibody specific for the CDK2 protein, a CDK2 protein-IP, or a CDK2 protein•CDK2 protein-IP complex, resistance to antibiotics, occlusion-body formation in baculovirus, and the like) caused by the insertion of the sequences of interest into the vector. In the third approach, recombinant expression vectors may be identified by assaying for the expression of the CDK2 protein concomitantly with expression of the aforementioned CDK2 protein-IPs by the recombinant vector.

Once the recombinant CDK2 protein and CDK2 protein-IP molecules have been identified and the complexes or individual proteins isolated, and a suitable host system and growth conditions have been established, the recombinant expression vectors may be propagated and amplified in-quantity. As previously discussed, expression vectors or their derivatives which can be used include, but are not limited to, human or animal viruses (*e.g.*, vaccinia virus or

adenovirus); insect viruses (*e.g.*, baculovirus); yeast vectors; bacteriophage vectors (*e.g.*, lambda phage); plasmid vectors and cosmid vectors.

A host cell strain may then be selected which modulates the expression of the inserted sequences of interest, or modifies/processes the expressed proteins in the specific manner desired. In addition, expression from certain promoters may be enhanced in the presence of certain inducers; thus facilitating control of the expression of the genetically-engineered CDK2 protein and/or CDK2 protein-IP. Moreover, different host cells possess characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation, and the like) of expressed proteins. Appropriate cell lines or host systems may thus be chosen to ensure the desired modification and processing of the foreign protein is achieved. For example, protein expression within a bacterial system can be used to produce an unglycosylated core protein; whereas expression within mammalian cells ensures "native" glycosylation of a heterologous protein.

In other specific embodiments, the CDK2 protein and/or CDK2 protein-IPs (or derivatives, fragments, analogs and homologs thereof) may be expressed as fusion or chimeric protein products comprising the protein joined via a peptide bond to a heterologous protein sequence of a different protein. Such chimeric products may be produced by the ligation of the appropriate nucleic acid sequences encoding the desired amino acids to one another in the proper coding frame and subsequently expressing the chimeric products in a suitable host by methods known within the art. Alternatively, such a chimeric product can be made by protein synthetic techniques (*e.g.*, by use of a peptide synthesizer). A specific embodiment of the present invention discloses a chimeric protein comprising a fragment of the CDK2 protein and/or a CDK2 protein-IP. In another specific embodiment, fusion proteins are provided which contain the domains of the CDK2 protein and a CDK2 protein-IP (which result in the direct formation of CDK2 protein•CDK2 protein-IP complexes) and, optionally, a heterofunctional reagent (*e.g.*, a peptide linker) which serves to both link the two aforementioned proteins and promote the interaction of the CDK2 protein and CDK2 protein-IP binding domains. These fusion proteins may be particularly useful where the stability of the interaction is desirable (*i.e.*, stability due to the formation of the complex as an intramolecular reaction), for example in production of antibodies specific to the CDK2 protein•CDK2 protein-IP complex.

In a specific embodiment of the present invention, the nucleic acids encoding proteins, and proteins consisting of, or comprising a fragment of the CDK2 protein or a CDK2 protein-IP which consists of at least 6 contiguous amino acid residues of the CDK2 protein and/or a CDK2 protein-IP, are provided herein. In another embodiment, the aforementioned protein fragment is comprised of at least 10, 20, 30, 40, or 50 amino acid residues (preferably not larger that 35, 100 or 200 amino acid residues) of the CDK2 protein or CDK2 protein-IP. Derivatives or analogs of the CDK2 protein and CDK2 protein-IPs include, but are not limited to, molecules comprising regions which are substantially homologous to the CDK2 protein or the CDK2 protein-IPs in various embodiments, of at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% amino acid identity when: (*i*) compared to an amino acid sequence of identical size; (*ii*) compared to an aligned sequence in which the alignment is done by a computer homology program known within the art or (*iii*) the encoding nucleic acid is capable of hybridizing to a sequence encoding the CDK2 protein or a CDK2 protein-IP under stringent, moderately stringent, or non-stringent conditions.

CDK2 protein and/or CDK2 protein-IP derivatives may be produced by alteration of their sequences by substitutions, additions or deletions which result in functionally-equivalent molecules. In a specific embodiment of the present invention, the degeneracy of nucleotide coding sequences allows for the use of other DNA sequences which encode substantially the same amino acid sequence as the CDK2 protein or CDK2 protein-IP genes. In another specific embodiment, one or more amino acid residues within the sequence of interest may be substituted by another amino acid of a similar polarity and net charge, thus resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. The CDK2 protein or CDK2 protein-IP derivatives and analogs of the present invention may be produced by various methodologies known within the art. For example, the cloned CDK2 protein and CDK2 protein-IP gene sequences may be modified by any of numerous methods known within the art. See *e.g.*, Sambrook, *et al.*, 1990. *Molecular Cloning: A Laboratory Manual, 2nd ed.*, (Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY). These sequences may be digested at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification, if so desired, and the resultant fragments isolated and ligated *in vitro*. Additionally, the CDK2 protein- or CDK2 protein-IP-encoding nucleic acids may be mutated *in vitro* or *in vivo* to: (*i*) create variations in coding

regions; (*ii*) create and/or destroy translation, initiation, and/or termination sequences and/or (*iii*) form new restriction endonuclease sites or destroy pre-existing ones, so as to facilitate further *in vitro* modification. Any technique for mutagenesis known within the art may be utilized, including but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (see *e.g.*, Hutchinson, *et al.*, 1978. *J. Biol. Chem 253*:6551-6558); by use of TABJ™ linkers (Pharmacia) and similar methodologies.

Once a recombinant cell expressing the CDK2 protein and/or a CDK2 protein-IP, or a fragment or derivative thereof, is identified, the individual gene product or complex may be isolated and analyzed. This is achieved by assays which are based upon the physical and/or functional properties of the protein or complex, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled products, and the like. The CDK2 protein•CDK2 protein-IP complexes may be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the proteins/protein complexes) including, but not limited to, column chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reverse-phase, high pressure, fast protein liquid, etc), differential centrifugation, differential solubility, or similar methodologies used for the purification of proteins. Alternatively, once CDK2 protein or CDK2 protein-IP or its derivative is identified, the amino acid sequence of the protein can be deduced from the nucleic acid sequence of the chimeric gene from which it was encoded. Hence, the protein or its derivative can be synthesized by standard chemical methodologies known in the art. See, *e.g.*, Hunkapiller, *et al.*, 1984. *Nature* 310:105-111.

In a specific embodiment of the present invention, such CDK2 protein•CDK2 protein-IP complexes, whether produced by recombinant DNA techniques, chemical synthesis methods or by purification from native sources, include, but are not limited to, those containing as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures 1-4 [SEQ ID NOS:2, 4, 6, and 8], as well as fragments, analogs and derivatives thereof, including proteins homologous thereto.

Manipulations of the CDK2 protein and/or CDK2 protein-IP sequences, may be made at the protein level. Included within the scope of the present invention are complexes of the CDK2 protein or CDK2 protein-IP fragments, derivatives, fragments or analogs which are differentially modified during or after translation (*e.g.*, by glycosylation, acetylation, phosphorylation,

amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, and the like). Any of the numerous chemical modification methodologies known within the art may be utilized including, but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc. In a specific embodiment, the CDK2 protein and/or CDK2 protein-IP sequences are modified to include a fluorescent label. In another specific embodiment, the CDK2 protein and/or the CDK2 protein-IP are modified by the incorporation of a heterofunctional reagent, wherein such heterofunctional reagent may be used to cross-link the members of the complex.

In addition, complexes of analogs and derivatives of the CDK2 protein and/or a CDK2 protein-IP can be chemically synthesized. For example, a peptide corresponding to a portion of the CDK2 protein and/or a CDK2 protein-IP, which comprises the desired domain or which mediates the desired activity *in vitro* (*e.g.*, CDK2 protein•CDK2 protein-IP complex formation), may be synthesized by use of a peptide synthesizer. In cases where natural products are suspected of being "mutant" or are isolated from new species, the amino acid sequence of the CDK2 protein, a CDK2 protein-IP isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, may be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. The CDK2 protein•CDK2 protein-IP complexes may also be analyzed by hydrophilicity analysis (see *e.g.*, Hopp & Woods, 1981. *Proc. Natl. Acad. Sci. USA* 78:3824-3828) which can be utilized to identify the hydrophobic and hydrophilic regions of the proteins, thus aiding in the design of substrates for experimental manipulation, such as in binding experiments, antibody synthesis, etc. Secondary structural analysis may also be performed to identify regions of the CDK2 protein and/or a CDK2 protein-IP which assume specific structural motifs. See *e.g.*, Chou & Fasman, 1974. *Biochem.* 13:222-223. Manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame prediction and plotting, and determination of sequence homologies, can be accomplished using computer software programs available in the art.

Other methods of structural analysis including, but not limited to, X-ray crystallography (see *e.g.*, Engstrom, 1974. *Biochem. Exp. Biol.* 11:7-13); mass spectroscopy and gas

chromatography (see *e.g.*, *Methods in Protein Science*, 1997. J. Wiley and Sons, New York, NY) and computer modeling (see *e.g.*, Fletterick & Zoller, eds., 1986. Computer Graphics and Molecular Modeling, *In: Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) may also be employed.

(2) Sequences Encoding hsReq*-1 and hsReq*-2

The present invention discloses the nucleotide sequences of nucleic acids which encode hsReq*-1 and hsReq*-2. In specific embodiments, the nucleic acid sequences of *hsReq*-1 and hsReq*-2* nucleic acids are set forth in SEQ ID NOS:10 and 12, respectively; wherein the associated inferred amino acid sequences of these nucleic acids are set forth in SEQ ID NOS:11 and 13, respectively. The present invention also relates to nucleic acids that are hybridizable or complementary to the aforementioned sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to (specifically, are the inverse complement of) at least 10, 25, 50, 100, or 200 nucleotides, or the entire coding region, of an *hsReq*-1* or *hsReq*-2* gene, that includes the portion of the *hsReq*-1* or *hsReq*-2* nucleotide sequence that spans the alternate splice junction (*i.e.*, not the splice junction formed in hsReq mRNA processing) of *hsReq*-1* or *hsReq*-2*.

In a specific embodiment of the present invention, a nucleic acid which is hybridizable to *hsReq*-1 or hsReq*-2* nucleic acids (*e.g.*, possessing a sequence which is anti-sense to SEQ ID NOS:10 or 12, respectively), or derivatives thereof, under conditions of low stringency hybridization is disclosed herein. By way of example, and not of limitation, procedures using such conditions of low, medium or high stringency hybridization can be as known to somebody skilled in the art (see *e.g.*, Shilo & Weinberg, 1981. *Proc. Natl. Acad. Sci. USA* 78:6789-6792). Other conditions of high stringency hybridization which well known within the art may also be utilized in the practice of the present invention.

Nucleic acids encoding derivatives, fragments and analogs of hsReq*-1 and hsReq*-2 proteins and *hsReq*-1 and hsReq*-2* antisense nucleic acids are additionally disclosed. The amino acid and nucleotide sequences for hsReq*-1 and hsReq*-2 were determined *in silico* as described above. Fragments of *hsReq*-1* or *hsReq*-2* nucleic acids comprising regions conserved between (with homology to) other *hsReq*-1* or *hsReq*-2* nucleic acids, of the same or different species, are also provided. Specifically, the invention relates to fragments of *hsReq*-1*

and *hsReq*-2* nucleic acids comprising a portion of the *hsReq*-1* or *hsReq*-2* nucleotide sequence that spans the alternate splice junction of *hsReq*-1* or *hsReq*-2*.

Regions within the 3' untranslated regions of the known protein cDNAs for *hsReq* were identified as encoding a protein or proteins that interact with CDK2 using the improved version of the yeast two hybrid system (*e.g.*, as described *infra*). The present inventors determined that the nucleotide sequences encoding the interacting proteins are identical to an untranslated portion of the *hsReq* nucleotide sequence from nucleotides 1789 to 2400 and from nucleotides 1819 to 2400 (as depicted in Figure 6 (SEQ ID NO:7)).

This indicates that hsReq*-1 and hsReq*-2 are encoded by mRNAs resulting from splicing of the unprocessed *hsReq* gene mRNA at splice sites other than the splice sites used in processing *hsReq* mRNA. These *hsReq*-1* and *hsReq*-2* sequences were determined by identifying alternate 5′ and 3′ splice sites in the *hsReq* sequence.

Determination of 5' and 3' splice points for protein splice variants can be performed by any method known in the art. For example, but not by way of limitation, the 5' and 3' splice points can be determined as follows:

(a) First, potential 5' splice sites can be identified in the coding sequence of the known protein, *i.e.*, hsReq. The sequence of 5' splice sites has an invariant GT sequence at the start of the intron, and the remaining bases are not invariant, but the preferred consensus sequence is AG:GTAAGT, with the colon indicating the splice point (Padgett *et al.*, 1984, Ann. Rev. Biochem. 55:1119-1150).

(b) Next, potential 3' intron:exon splice sites can also be identified based on the consensus analysis described by Padgett et al. (1984, Ann. Rev. Biochem. 55:1119-1150). The 3' intron:exon splice site must have an AG sequence 5' to the splice site (denoted as "AG:") and the base 5' to (preceding) the AG: sequence must be a C or a T. The nucleotides 5 to 14 nucleotides 5' of the last G nucleotide of the intron can contain at most two non-T, non-C bases (Padgett *et al.*, 1984, Ann. Rev. Biochem. 55:1119-1150). To identify such a potential 3' intron:exon splice site, the sequence between a potential 5' splice site and the start of the nucleotide sequence encoding the detected interacting protein or protein fragment

is scanned for the invariant AG: sequence. where the base preceding the invariant region must be a C or T.

(c) Next. based on the known translational frame of the mature protein and each predicted 5' splice site. compatible translational frames for successful splicing are defined for potential 3' splice sites. Nucleotide sequences can be analyzed by a number of nucleotide sequence analysis programs available in the art to define possible protein translation products. Translation in the three forward translation frames defines possible open reading frames (contiguous spans of codons for amino acids without the presence of a stop codon). Only those 3' sites that match the necessary translational frame of a 5' prime splice junction are retained. Unmatched 5' or 3' splice sites are eliminated. In cases where no ideal 3' splice site match is found. sites containing three non-C. non-T bases upstream of the splice site are then examined.

(d) For each possible 5':3' splice site pair, a search for a mammalian branch point consensus sequence is performed (Reed and Maniatis, 1988. Genes Dev. 2:1268-1276). The branch point is identified by the consensus sequence T/CNCTGAC to which 5 of the 6 defined bases must match and the consensus sequence must be 20-60 nucleotides 5' of the 3' splice site. Though not absolutely required for pre mRNA splicing, the presence of the consensus sequence increases splicing efficiency. Thus, 5':3' splice site pairs with a branch point consensus sequence are retained over splice site pairs that do not have a branch point consensus sequence.

(e) Finally, new splice variant proteins must encode at least 60 amino acid residues to constitute a viable *in vivo* product. Further. the 3' end of slice variants must, by definition, extend into the identified interacting sequence.

The amino acid and nucleotide sequences for two splice variants of hsReq, named hsReq*-1 and hsReq*-2 in this invention and depicted in Figures 6 and 7, respectively, were determined *in silico* as described above and as exemplified in Section 6.3 *infra*. For *hsReq*-1*, a 5' splice site was identified at nucleotides 563-570 of the *hsReq* nucleotide sequence (Figure 4),

with the last base of the first exon being nucleotide number 564. and a 3' splice site was identified at nucleotides 1566 to 1580 of the *hsReq* nucleotide sequence. with the first base of the second exon being nucleotide number 1580. The translation stop codon of *hsReq*-1* was identified as nucleotides 1861 to 1863 of the *hsReq* nucleotide sequence. The branch point consensus region for *hsReq*-1* splicing was identified at nucleotides 1538 to 1544 of the *hsReq* nucleotide sequence.

For *hsReq*-2*, a 5' splice site was identified at nucleotides 563-570 of the *hsReq* sequence (Figure 4), with the last base of the first exon being nucleotide number 564, and a 3' splice site was identified at nucleotides 1776-1790 of the *hsReq* nucleotide sequence, with the last base of the second exon being nucleotide number 1790. The branch point site associated with this 3' splice site is at nucleotides 1759 to 1765 of the *hsReq* sequence, and the translation stop codon for hsReq*-2 is nucleotides 1861 to 1863 of the *hsReq* nucleotide sequence.

Any methodology available within the art may be utilized to obtain a full-length (*i.e.*, encompassing the entire coding region) cDNA clone encoding *hsReq*-1 and hsReq*-2*. For example, the polymerase chain reaction (PCR) may be utilized to amplify the sequence within a cDNA library. Similarly, oligonucleotide primers may also be used to amplify by PCR sequences from a nucleic acid sample (RNA or DNA), preferably a cDNA library, from an appropriate source (*e.g.*, the sample from which the initial cDNA library for the modified yeast two hybrid assay fusion population was derived).

PCR may be performed by use of, for example, a Perkin-Elmer Cetus thermal cycler and *Taq* polymerase. The DNA being amplified is preferably cDNA derived from any eukaryotic species. It should be noted that several different degenerate primers may be synthesized for use in the PCR reactions. It is also possible to vary the stringency of the hybridization conditions used in priming the PCR reactions, to amplify nucleic acid homologs by allowing for greater or lesser degrees of nucleotide sequence similarity between the known nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred; whereas for same species hybridization, moderately stringent conditions are preferred.

Any eukaryotic cell may potentially serve as the nucleic acid source for the molecular cloning of the *hsReq*-1 and hsReq*-2* sequences. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by

cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. See *e.g.*, Sambrook, *et al.*, 1989. *Molecular Cloning: A Laboratory Manual. 2nd ed.*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); Glover, 1985. *DNA Cloning: A Practical Approach* (MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intronic DNA regions in addition to exonic (coding) regions; whereas clones derived from cDNA will contain only exonic sequences.

In a preferable embodiment of the present invention, *hsReq*-1 and hsReq*-2* nucleic acids are derived from a cDNA source. Identification of the specific cDNA containing the desired sequence may be accomplished in a number of ways. In one methodology, a portion of the hsReq*-1 or hsReq*-2sequence (*e.g.*, a PCR amplification product obtained as described *supra*), or an oligonucleotide possessing a sequence of a portion of the known nucleotide sequence, or its specific RNA, or a fragment thereof, may be purified, amplified, and labeled, and the generated nucleic acid fragments may be screened by nucleic acid hybridization utilizing a labeled probe. See *e.g.*, Benton & Davis, 1977. *Science* 196:180. In a second methodology, the appropriate fragment is identified by restriction enzyme digestion(s) and comparison of fragment sizes with those expected from comparison to a known restriction map (if such is available) or by DNA sequence analysis and comparison to the known nucleotide sequence of *hsReq*-1 and hsReq*-2*. In a third methodology, the gene of interest may be detected utilizing assays based on the physical, chemical or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, may be selected as a function of their production of a protein which, for example, has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, antigenic properties or ability to bind the CDK2 protein. In a fourth methodology, should an anti-hsReq*-1 or anti-hsReq*-2antibody be available, the protein of interest may be identified by the binding of a labeled antibody to the putatively hsReq*-1 and hsReq*-2 clone in an enzyme-linked immunosorbent assay (ELISA).

In specific embodiments of the present invention, following isolation and identification, the nucleic acids may then be inserted into an appropriate cloning vector including, but are not limited to, bacteriophages (*e.g.*, λ derivatives) or bacterial plasmids (*e.g.*, pBR322, pUC, or the Bluescript® vector (Stratagene; La Jolla, CA). The insertion of the nucleic acid of interest into a cloning vector may be facilitated by, for example, ligating the DNA fragment into a vector

possessing complementary cohesive termini or, if there are no complementary cohesive termini present in the cloning vector, the termini of the DNA insert or vector molecule may be enzymatically modified. Alternatively, any restriction site may be produced by the ligation of linker sequences onto the DNA termini; wherein these linker sequences may comprise specific chemically-synthesized oligonucleotides possessing restriction endonuclease recognition sequences. In an additional embodiment, both the cleaved vector and hsReq*-1 and hsReq*-2 sequence may be modified by complementary, homopolymeric tailing. Recombinant molecules may be introduced into host cells via transformation, transfection, infection, electroporation, and the like. In yet another embodiment, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shotgun" approach. Enrichment for the desired gene (*e.g.*, by size fractionation) may be done before insertion into the cloning vector.

The *hsReq*-1 and hsReq*-2* sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native hsReq*-1 and hsReq*-2 proteins, and those encoded amino acid sequences with functionally equivalent amino acids, as well as those encoding other hsReq*-1 and hsReq*-2 derivatives, fragments or analogs.

(3) Production of Antibodies to CDK2 Protein•CDK2 Protein-IP Complexes

As disclosed by the present invention herein, CDK2 protein•CDK2 protein-IP complexes, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} fragments and an F_{ab} expression library. In a specific embodiment, antibodies to complexes of human CDK2 protein and human CDK2 protein-IP are disclosed. In another specific embodiment, complexes formed from fragments of the CDK2 protein and a CDK2 protein-IP; wherein these fragments contain the protein domain which interacts with the other member of the complex and are used as immunogens for antibody production. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a CDK2 protein•CDK2 protein-IP complex, or derivative, fragment, analog or homolog thereof.

For the production of polyclonal antibodies, various host animals may be immunized by injection with the native CDK2 protein•CDK2 protein-IP complex, or a synthetic version, or a derivative of the foregoing (*e.g.*, a cross-linked CDK2 protein•CDK2 protein-IP). Various adjuvants may be used to increase the immunological response and include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.) and human adjuvants such as *Bacille Calmette-Guerin* (BCG) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed towards a CDK2 protein•CDK2 protein-IP complex, or derivatives, fragments, analogs or homologs thereof, any technique which provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975. *Nature* 256:495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983. *Immunol. Today* 4:72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the present invention, monoclonal antibodies may be produced in germ-free animals utilizing recently developed technology. See PCT Publication US 90/02545. Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by the use of human hybridomas (see Cote, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:2026-2030) or by transforming human B-cells with Epstein Barr Virus (EBV) *in vitro* (see Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96).

In an additional embodiment of the present invention, techniques are disclosed for the production of single-chain antibodies (see *e.g.*, U.S. Patent No. 4,946,778) may be adapted for the production of CDK2 protein•CDK2 protein-IP complex-specific single-chain antibodies. In yet another embodiment, methodologies are disclosed for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989. *Science* 246:1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for CDK2 protein•CDK2 protein-IP or derivatives, fragments, analogs or homologs thereof. Furthermore, the present invention discloses methodologies for the "humanization" of non-human antibodies by techniques known within the art. See *e.g.*, U.S. Patent No. 5,225,539). Antibody fragments

which contain the idiotypes of CDK2 protein•CDK2 protein-IP complexes may be produced by techniques known in the art including, but not limited to: (*i*) the $F(ab')_2$ fragment which is produced by pepsin digestion of an antibody molecule; (*ii*) the Fab fragments which may be generated by the reduction of the disulfide bridges of the $F(ab')_2$ fragment; (*iii*) the F_{ab} fragments which may be generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

In one embodiment of the present invention, methodologies for the screening of antibodies which possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies which are specific to a particular domain of the CDK2 protein•CDK2 protein-IP complex is facilitated by generation of hybridomas which binds to the fragment of the CDK2 protein•CDK2 protein-IP complex possessing such a domain. In another specific embodiment, methodologies for the selection of an antibody which specifically-binds a CDK2 protein•CDK2 protein-IP complex but which does not specifically-bind to the individual proteins of the CDK2 protein•CDK2 protein-IP complex (by selecting the antibody on the basis of positive-binding to the CDK2 protein•CDK2 protein-IP complex with a concomitant lack of binding to the individual CDK2 protein and CDK2 protein-IP proteins) are disclosed herein. Accordingly, antibodies which are specific for a domain within the CDK2 protein•CDK2 protein-IP complex, or derivative, fragments, analogs or homologs thereof, are also provided herein.

It should be noted that the aforementioned antibodies may be used in methods known within the art relating to the localization and/or quantitation of CDK2 protein•CDK2 protein-IP complexes (*e.g.*, for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In yet another embodiment of the present invention, anti-CDK2 protein•CDK2 protein-IP complex antibodies, or derivatives, fragments, analogs or homologs thereof, which possess the protein binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

(4) Use of CDK2 Protein•CDK2 Protein-IP Complexes in Diagnosis, Prognosis and Screening

CDK2 protein•CDK2 protein-IP complexes (*i.e.*, particularly the CDK2 protein complexed with cyclin I, ERH, hsReq*-1 and hsReq*-2), may serve as "markers" for specific disease states which involve the disruption of physiological processes including, but not limited to: (*i*) control of cell-cycle progression; (*ii*) cellular differentiation and apoptosis; (*iii*) regulation of transcription; (iv) pathological processes including, but not restricted to, hyperproliferative disorders (*e.g.*, tumorigenesis and tumor progression); and atherosclerosis, and thus may have diagnostic utility. In accord, the differentiation and classification of particular groups of patients possessing elevations or deficiencies of a CDK2 protein•CDK2 protein-IP complex may lead to new nosological classifications of diseases, thus markedly advancing diagnostic ability.

The detection of CDK2 protein•CDK2 protein-IP complex levels, or the levels of the individual proteins which have been shown to form complexes with the CDK2 protein, or detecting the levels of the mRNAs which encode the components of the CDK2 protein•CDK2 protein-IP complexes, may be utilized in diagnosis, prognosis, following the disease course, following the efficacy of administered therapeutics, of disease states, following therapeutic response, etc. Similarly, both the nucleic acid sequences (and sequences complementary thereto) and anti-CDK2 protein•CDK2 protein-IP complex antibodies and antibodies directed against the individual components that can form CDK2 protein•CDK2 protein-IP complexes, have uses in diagnostics. Such molecules may be utilized in assays (*e.g.*, immunoassays) to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders characterized by aberrant levels of CDK2 protein•CDK2 protein-IP complexes, or monitor the treatment thereof. The aforementioned immunoassay may be performed by a methodology comprising contacting a sample derived from a patient with an anti-CDK2 protein•CDK2 protein-IP complex antibody under conditions such that immunospecific-binding may occur, and subsequently detecting or measuring the amount of any immunospecific-binding by the antibody. In a specific embodiment, an antibody specific for a CDK2 protein•CDK2 protein-IP complex may be used to analyze a tissue or serum sample from a patient for the presence of CDK2 protein•CDK2 protein-IP complex; wherein an aberrant level of CDK2 protein•CDK2 protein-IP complex is indicative of a diseased condition. The immunoassays which may be utilized include, but are not limited to, competitive and non-competitive assay systems using techniques such as Western

Blots, radioimmunoassays (RIA), enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein-A immunoassays, etc.

The nucleic acid species of the present invention encoding the associated protein components of the CDK2 protein•CDK2 protein-IP complexes, and related nucleotide sequences and subsequences, may also be used in hybridization assays. The CDK2 protein and CDK2 protein-IP nucleotide sequences, or subsequences thereof comprising at least 8 nucleotides, may be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant levels of the mRNAs encoding the components of a CDK2 protein•CDK2 protein-IP complex, as described *supra*. In specific embodiments of the present invention, diseases and disorders involving or characterized by aberrant levels of CDK2 protein•CDK2 protein-IP complexes or a predisposition to develop such disorders may be diagnosed by detecting aberrant levels of CDK2 protein•CDK2 protein-IP complexes, or non-complexed CDK2 protein and/or CDK2 protein-IP proteins or nucleic acids for functional activity. This aforementioned functional activity may including, but is not restricted to, (*i*) binding to an interacting partner (*e.g.*, the CDK2 protein, cyclin I, ERH, hsReq*-1 and hsReq*-2) or (*ii*) by detecting mutations in CDK2 protein and/or a CDK2 protein-IP RNA, DNA or protein (*e.g.*, translocations, truncations, changes in nucleotide or amino acid sequence relative to wild-type CDK2 protein and/or the CDK2 protein-IP) which can cause increased or decreased expression or activity of the CDK2 protein, a CDK2 protein-IP or a CDK2 protein• :CDK2 protein-IP complex.

Methodologies which are well-known within the art (*e.g.*, immunoassays, nucleic acid hybridization assays, biological activity assays, and the like) may be used to determine whether one or more particular CDK2 protein•CDK2 protein-IP complexes are present at either increased or decreased levels, or are absent, within samples derived from patients suffering from a particular disease or disorder, or possessing a predisposition to develop such a disease or disorder, as compared to the levels in samples from subjects not having such disease or disorder or predisposition thereto. Additionally, these assays may be utilized to determine whether the ratio of the CDK2 protein•CDK2 protein-IP complex to the non-complexed components (*i.e.* the CDK2 protein and/or the specific CDK2 protein-IP) in the complex of interest is increased or

decreased in samples from patients suffering from a particular disease or disorder or having a predisposition to develop such a disease or disorder as compared to the ratio in samples from subjects not having such a disease or disorder or predisposition thereto.

Accordingly, in specific embodiments of the present invention, diseases and disorders which involve increased/decreased levels of one or more CDK2 protein•CDK2 protein-IP complexes may be diagnosed, or their suspected presence may be screened for, or a predisposition to develop such diseases and disorders may be detected, by quantitatively ascertaining increased/decreased levels of: (*i*) the one or more CDK2 protein•CDK2 protein-IP complexes; (*ii*) the mRNA encoding both protein members of said complex; (*iii*) the complex functional activity or (*iv*) mutations in the CDK2 protein or the CDK2 protein-IP (*e.g.*, translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type CDK2 protein or the CDK2 protein-IP) which enhance/inhibit or stabilize/destabilize CDK2 protein•CDK2 protein-IP complex formation.

In the practice of the present invention, the use of detection techniques, especially those involving antibodies directed against the CDK2 protein•CDK2 protein-IP complexes, provide methods for the detection of specific cells which express the protein or protein complex of interest. Using such assays, specific cell types may be quantitatively characterized in which one or more particular CDK2 protein•CDK2 protein-IP complex are expressed, and the presence of the protein or protein complex may be correlated with cell viability by techniques well-known within the art (*e.g.*, florescence-activated cell sorting). Also embodied herein are methodologies directed to the detection of a CDK2 protein•CDK2 protein-IP complex within *in vitro* cell culture models which express particular CDK2 protein•CDK2 protein-IP complexes, or derivatives thereof, for the purpose of characterizing and/or isolating CDK2 protein•CDK2 protein-IP complexes. These detection techniques include, but are not limited to, cell-sorting of prokaryotes (see *e.g.*, Davey & Kell, 1996. *Microbiol. Rev.* 60:641-696); primary cultures and tissue specimens from eukaryotes, including mammalian species such as human (see *e.g.*, Steele, *et al.*, 1996. *Clin. Obstet. Gynecol.* 39:801-813) and continuous cell cultures (see *e.g.*, Orfao & Ruiz-Arguelles, 1996. *Clin. Biochem.* 29:5-9.

The present invention additionally provides kits for diagnostic use which are comprised of one or more containers containing an anti-CDK2 protein•CDK2 protein-IP complex antibody and, optionally, a labeled binding partner to said antibody. The label incorporated into the anti-

CDK2 protein•CDK2 protein-IP complex antibody may include, but is not limited to, a chemiluminescent, enzymatic, fluorescent, colorimetric or radioactive moiety. In an alternative specific embodiment, the kit may comprise, in one or more containers, a pair of oligonucleotide primers (*e.g.*, each 6-30 nucleotides in length) which are capable of acting as amplification primers for: polymerase chain reaction (PCR; see *e.g.*, Innis, *et al.*, 1990. *PCR Protocols* (Academic Press, Inc., San Diego, CA)); ligase chain reaction; cyclic probe reaction, or other methods known within the art. The kit may, optionally, further comprise a predetermined amount of a purified CDK2 protein, CDK2 protein-IP or CDK2•CDK2 protein-IP complex, or nucleic acids thereof, for use as a standard or control in the aforementioned assays.

(5) Therapeutic Uses of the CDK2 Protein, CDK2 Protein-IP and CDK2 Protein•CDK2 Protein-IP Complexes

The present invention provides for treatment or prevention of various diseases and disorders by administration of a biologically-active, therapeutic compound (hereinafter "Therapeutic"). Such Therapeutics include, but are not limited to: (*i*) various CDK2 protein• CDK2 protein-IP complexes (*e.g.*, the CDK2 protein complexed with cyclin I, ERH, hsReq*-1 and hsReq*-2) and derivative, fragments, analogs and homologs thereof; (*ii*) antibodies directed against the aforementioned proteins and protein complexes thereof; (*iii*) nucleic acids encoding the CDK2 protein and CDK2 protein-IPs and derivatives, fragments, analogs and homologs thereof; (*iv*) antisense nucleic acids encoding the CDK2 protein and (*v*)CDK2 protein IPs and CDK2 protein•CDK2 protein-IP complex and modulators (*i.e.*, inhibitors, agonists and antagonists) thereof.

As previously discussed, the CDK2 protein has been implicated to play a significant role in disorders of cell-cycle progression, cell differentiation, and transcriptional control, including cancer and tumorigenesis and tumor progression. Atherosclerosis may also involve the CDK2 protein and/or CDK2 protein-IPs.

(*i*) Disorders with Increased CDK2 protein and CDK2 protein•CDK2 protein-IP Complex Levels

Diseases and disorders which are characterized by increased (relative to a subject not suffering from said disease or disorder) CDK2 protein•CDK2 protein-IP levels or biological activity may be treated with Therapeutics which antagonize (*i.e.*, reduce or inhibit) CDK2 protein

•CDK2 protein-IP complex formation or activity. Therapeutics which antagonize CDK2 protein •CDK2 protein-IP complex formation or activity may be administered in a therapeutic or prophylactic manner. Therapeutics which may be utilized include, but are not limited to, the CDK2 protein or CDK2 protein-IPs, or analogs, derivatives, fragments or homologs thereof; (*ii*) anti-CDK2 protein•CDK2 protein-IP complex antibodies; (*iii*) nucleic acids encoding the CDK2 protein or a CDK2 protein-IP; (*iv*) concurrent administration of a CDK2 protein and a CDK2 protein-IP antisense nucleic acid and CDK2 protein and/or CDK2 protein-IP nucleic acids which are "dysfunctional" (*i.e.*, due to a heterologous [non-CDK2 protein and/or non-CDK2 protein-IP] insertion within the coding sequences of the CDK2 protein and CDK2 protein-IP coding sequences) are utilized to "knockout" endogenous CDK2 protein and/or CDK2 protein-IP function by homologous recombination (see *e.g.*, Capecchi, 1989. *Science* 244:1288-1292). In an additionally embodiment of the present invention, mutants or derivatives of a first CDK2 protein-IP which possess greater affinity for CDK2 protein than the wild-type first CDK2 protein-IP may be administered to compete with a second CDK2 protein-IP for binding to the CDK2 protein, thereby reducing the levels of complexes between the CDK2 protein and the second CDK2 protein-IP.

Increased levels of CDK2 protein•CDK2 protein-IP complexes can be readily detected by quantifying protein and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or protein levels, structure and/or activity of the expressed CDK2 protein•CDK2 protein-IP complex (or the CDK2 protein and CDK2 protein-IP mRNAs). Methods which are well-known within the art including, but not limited to, immunoassays to detect CDK2 protein•CDK2 protein-IP complexes (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect concurrent expression of the CDK2 protein and a CDK2 protein-IP mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

(*ii*) Disorders with Increased CDK2 protein and CDK2 protein•CDK2 protein-IP Complex Levels

A specific embodiment of the present invention discloses methods for the reduction of CDK2 protein•CDK2 protein-IP complex expression (*i.e.*, the expression of the two protein

components of the complex and/or formation of the complex) by targeting mRNAs which express the protein moieties. RNA Therapeutics are, currently, differentiated into three classes: (*i*) antisense species; (*ii*) ribozymes or (*iii*) RNA aptamers. See *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4:45-54. Antisense oligonucleotides have been the most widely utilized and will be discussed, *infra*. Ribozyme therapy involves the administration (*i.e.*, induced expression) of small RNA molecules with enzymatic ability to cleave, bind, or otherwise inactivate specific RNAs, thus reducing or eliminating the expression of particular proteins. See *e.g.*, Grassi & Marini, 1996. *Ann. Med.* 28:499-510. At present, the design of "hairpin" and/or "hammerhead" RNA ribozymes are necessary to specifically-target a particular mRNA (*e.g.*, the CDK2 protein mRNA). RNA aptamers are specific RNA ligands for proteins, such as for *Tat* and *Rev* RNA (see *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4:45-54) which can specifically inhibit their translation.

In a preferred embodiment of the present invention, the activity or level of the CDK2 protein may be reduced by administration of a CDK2 protein-IP, a nucleic acid which encodes the CDK2 protein-IP or an antibody (or a derivative or fragment of the antibody possessing the binding domain thereof) which immunospecifically-binds to the CDK2 protein-IP. Similarly, the levels or activity of a CDK2 protein-IP may be reduced by administration of the CDK2 protein, a nucleic acid encoding the CDK2 protein or an antibody (or a derivative or fragment of the antibody possessing the binding domain thereof) which immunospecifically-binds the CDK2 protein. In another embodiment of the present invention, diseases or disorders which are associated with increased levels of the CDK2 protein, or a particular CDK2 protein-IP, may be treated or prevented by administration of a Therapeutic which increases CDK2 protein•CDK2 protein-IP complex formation, if said complex formation acts to reduce or inactivate the CDK2 protein or the particular CDK2 protein-IP via CDK2 protein•CDK2 protein-IP complex formation. Such diseases or disorders may be treated or prevented by: (*i*) the administration of one member of the CDK2 protein•CDK2 protein-IP complex, including mutants of one or both of the proteins which possess increased affinity for the other member of the CDK2 protein• CDK2 protein-IP complex (so as to cause increased complex formation) or (*ii*) the administration of antibodies or other molecules which serve to stabilize the CDK2 protein•CDK2 protein-IP complex, or the like.

(6) Determination of the Biological Effect of the Therapeutic

In preferred embodiments of the present invention, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon said cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

(*i*) Malignancies

Components of the CDK2 protein•CDK2 protein-IP complexes (*i.e.*, the CDK2 protein, cyclin I, ERB, hsReq*-1 and hsReq*-2) are involved in the regulation of cell proliferation. Accordingly, Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of diseases or disorders which are associated with cell hyperproliferation and/or loss of control of cell proliferation (*e.g.*, cancers, malignancies and tumors). For a review of such hyperproliferation disorders, see *e.g.*, Fishman, *et al.*, 1985. *Medicine*, 2nd *ed.* (J.B. Lippincott Co., Philadelphia, PA).

Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assay include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from the patient's tumor, as well as *in vivo* assays using animal models of cancer or malignancies. Potentially effective Therapeutics, for example, inhibit the proliferation of tumor-derived or transformed cells in culture or cause a regression of tumors in animal models, in comparison to the controls.

In the practice of the present invention, once a malignancy or cancer has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) CDK2 protein•CDK2 protein-IP complex activity, that cancer or malignancy may subsequently be treated or prevented by the administration of a Therapeutic which serves to modulate CDK2 protein•CDK2 protein-IP complex formation and function, including supplying CDK2 protein•

CDK2 protein-IP complexes and the individual binding partners of said protein complex (*i.e.*, the CDK2 protein and/or a CDK2 protein protein-IP.

(*ii*) Pre-Malignant Conditions

The Therapeutics of the present invention which are effective in the therapeutic or prophylactic treatment of cancer or malignancies may also be administered for the treatment of pre-malignant conditions and/or to prevent the progression of a pre-malignancy to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia or, most particularly, dysplasia has occurred. For a review of such abnormal cell growth see *e.g.*, Robbins & Angell, 1976. *Basic Pathology, 2nd ed.* (W.B. Saunders Co., Philadelphia, PA). Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in its structure or function. For example, it has been demonstrated that endometrial hyperplasia often precedes endometrial cancer.

Metaplasia is a form of controlled cell growth in which one type of mature or fully differentiated cell substitutes for another type of mature cell. Metaplasia may occur in epithelial or connective tissue cells; whereas atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is generally considered a precursor of cancer, and is found mainly in the epithelia. Dysplasia is the most disorderly form of non-neoplastic cell growth, and involves a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed or malignant phenotype displayed either *in vivo* or *in vitro* within a cell sample derived from a patient, is indicative of the desirability of prophylactic/therapeutic administration of a Therapeutic of the present invention which possesses the ability to modulate CDK2 protein• CDK2 protein-IP complex activity. Characteristics of a transformed phenotype include, but are not limited to: (*i*) morphological changes; (*ii*) looser substratum attachment; (*iii*) loss of cell-to-

cell contact inhibition; (*iv*) loss of anchorage dependence; (*v*) protease release; (*vi*) increased sugar transport; (*vii*) decreased serum requirement; (*viii*) expression of fetal antigens; (*ix*) disappearance of the 250 Kdal cell-surface protein and the like. See *e.g.*, Richards, *et al.*, 1986. *Molecular Pathology* (W.B. Saunders Co., Philadelphia, PA).

In a specific embodiment of the present invention, leukoplakia (a benign-appearing hyperplastic or dysplastic lesion of the epithelium) or Bowen's disease (a carcinoma *in situ*) are pre-neoplastic lesions which are illustrative of the desirability of prophylactic intervention to prevent transformation to a frankly malignant phenotype. In another specific embodiment, the Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of fibrocystic diseases including, but not limited to, cystic hyperplasia, mammary dysplasia and, particularly, adenosis (benign epithelial hyperplasia).

In other preferred embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: (*i*) a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome (bcr/*abl*) for chronic myelogenous leukemia and t(14;18) for follicular lymphoma, etc.); (*ii*) familial polyposis or Gardner's syndrome (possible forerunners of colon cancer); (*iii*) monoclonal gammopathy of undetermined significance (MGUS; a possible precursor of multiple myeloma) and (*iv*) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia and Bloom's syndrome).

In another preferred embodiment, a Therapeutic of the present invention is administered to a human patient to prevent the progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

(*iii*) Hyperproliferative and Dysproliferative Disorders

In a preferred embodiment of the present invention, a Therapeutic is administered in the therapeutic or prophylactic treatment of hyperproliferative or benign dysproliferative disorders.

The efficacy in treating or preventing hyperproliferative diseases or disorders of a Therapeutic of the present invention may be assayed by any method known within the art. Such assays include *in vitro* cell proliferation assays, *in vitro* or *in vivo* assays using animal models of hyperproliferative diseases or disorders, or the like. Potentially effective Therapeutics may, for example, promote cell proliferation in culture or cause growth or cell proliferation in animal models in comparison to controls.

In accord, once a hyperproliferative disorder has been shown to be amenable to treatment by modulation of CDK2 protein•CDK2 protein-IP complex activity, the hyperproliferative disease or disorder may be treated or prevented by the administration of a Therapeutic which modulates CDK2 protein•CDK2 protein-IP complex formation (including supplying CDK2 protein•CDK2 protein-IP complexes and the individual binding partners of a CDK2 protein• CDK2 protein-IP complex (*e.g.*, the CDK2 protein, cyclin I, ERH, hsReq*-1 and hsReq*-2).

Specific embodiments of the present invention are directed to the treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid (hypertrophic scar) formation causing disfiguring of the skin in which the scarring process interferes with normal renewal; psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination); benign tumors; fibrocystic conditions and tissue hypertrophy (*e.g.*, benign prostatic hypertrophy).

(*iv*) Atherosclerosis

The CDK2 protein plays a role in the regulation of atherosclerosis. Accordingly, Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of atherosclerotic diseases or disorders. Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing atherosclerosis and related disorders. Such assays include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from atherosclerotic plaques, as well as *in vivo* assays using animal models of atherosclerosis. Potentially effective Therapeutics, for example, inhibit the inflammatory activity in human atherosclerotic plaques, in comparison to the controls.

In the practice of the present invention, once atherosclerosis has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) CDK2 protein• CDK2 protein-IP complex activity, that atherosclerosis may subsequently be treated or prevented

by the administration of a Therapeutic which serves to modulate CDK2 protein•CDK2 protein-IP complex formation and function, including supplying CDK2 protein•:CDK2 protein-IP complexes and the individual binding partners of said protein complex (*i.e.*, the CDK2 protein and/or a CDK2 protein-IP.

(7) Gene Therapy

In a specific embodiment of the present invention, nucleic acids comprising a sequence which encodes the CDK2 protein and/or a CDK2 protein-IP, or functional derivatives thereof, are administered to modulate CDK2 protein•CDK2 protein-IP complex function, by way of gene therapy. In more specific embodiments, a nucleic acid or nucleic acids encoding both the CDK2 protein and a CDK2 protein-IP (*e.g.*, cyclin I, ERH, hsReq*-1 and hsReq*-2), or functional derivatives thereof, are administered by way of gene therapy. Gene therapy refers to therapy that is performed by the administration of a specific nucleic acid to a subject. In this embodiment of the present invention, the nucleic acid produces its encoded protein(s), which then serve to exert a therapeutic effect by modulating CDK2 protein•CDK2 protein-IP complex function. Any of the methodologies relating to gene therapy available within the art may be used in the practice of the present invention. See *e.g.*, Goldspiel, *et al.*, 1993. *Clin. Pharm.* 12:488-505.

In a preferred embodiment, the Therapeutic comprises a CDK2 protein and a CDK2 protein-IP nucleic acid that is part of an expression vector expressing both of the aforementioned proteins, or fragments or chimeric proteins thereof, within a suitable host. In a specific embodiment, such a nucleic acid possesses a promoter which is operably-linked to the CDK2 protein and the CDK2 protein-IP coding region(s), or, less preferably two separate promoters linked to the CDK2 protein and the CDK2 protein-IP coding regions separately; wherein said promoter is inducible or constitutive, and, optionally, tissue-specific. In another specific embodiment, a nucleic acid molecule is used in which the CDK2 protein and CDK2 protein-IP coding sequences (and any other desired sequences) are flanked by regions which promote homologous recombination at a desired site within the genome, thus providing for intra-chromosomal expression of the CDK2 protein and the CDK2 protein-IP nucleic acids. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86:8932-8935.

Delivery of the Therapeutic nucleic acid into a patient may be either direct (*i.e.*, the patient is directly exposed to the nucleic acid or nucleic acid-containing vector) or indirect (*i.e.*,

cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient). These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy. In a specific embodiment of the present invention, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This may be accomplished by any of numerous methods known in the art including, but not limited to: (*i*) constructing it as part of an appropriate nucleic acid expression vector and administering in a manner such that it becomes intracellular (*e.g.*, by infection using a defective or attenuated retroviral or other viral vector ; see U.S. Patent No. 4,980,286) or (*ii*) direct injection of naked DNA, or through the use of microparticle bombardment (*e.g.*, a "Gene Gun®; Biolistic, DuPont), or by coating it with lipids, cell-surface receptors/transfecting agents, or through encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, or by administering it in linkage to a ligand predisposed to receptor-mediated endocytosis (see *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432), which can be used to "target" cell types which specifically express the receptors of interest, etc.

In another specific embodiment of the present invention, a nucleic acid-ligand complex may be produced in which the ligand comprises a fusogenic viral peptide designed so as to disrupt endosomes, thus allowing the nucleic acid to avoid subsequent lysosomal degradation. In yet another specific embodiment, the nucleic acid may be targeted *in vivo* for cell-specific endocytosis and expression, by targeting a specific receptor. See *e.g.*, PCT Publications WO 92/06180; WO93/14188 and WO 93/20221. Alternatively, the nucleic acid may be introduced intracellularly and incorporated within host cell genome for expression by homologous recombination. See *e.g.*, Zijlstra, *et al.*, 1989. *Nature* 342:435-438.

In yet another specific embodiment, a viral vector which contains the CDK2 protein and/or the CDK2 protein-IP nucleic acids is utilized. For example, retroviral vectors may be employed (see *e.g.*, Miller, *et al.*, 1993. *Meth. Enzymol.* 217:581-599) which have been modified to delete those retroviral-specific sequences which are not required for packaging of the viral genome and its subsequent integration into host cell DNA. The CDK2 protein and/or CDK2 protein-IP (preferably both protein species) nucleic acids are cloned into the vector, which facilitates delivery of the genes into a patient. See *e.g.*, Boesen, *et al.*, 1994. *Biotherapy* 6:291-302; Kiem, *et al.*, 1994. *Blood* 83:1467-1473. Additionally, adenovirus is an especially efficacious "vehicle" for the delivery of genes to the respiratory epithelia. Other targets for

adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses also possess the advantageous ability to infect non-dividing cells. For a review see *e.g.*, Kozarsky & Wilson, 1993. *Curr. Opin. Gen. Develop.* 3:499-503. Adenovirus-associated virus (AAV) has also been proposed for use in gene therapy. See *e.g.*, Walsh, *et al.*, 1993. *Proc. Soc. Exp. Biol. Med.* 204:289-300.

An additional approach to gene therapy in the practice of the present invention involves transferring a gene into cells in *in vitro* tissue culture by such methods as electroporation, lipofection, calcium phosphate-mediated transfection, or viral infection. Generally, the methodology of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection pressure (*e.g.*, antibiotic resistance) so as facilitate the isolation of those cells which have taken up, and are expressing the transferred gene. Those cells are then delivered to a patient. In this specific embodiment, the nucleic acid is introduced into a cell prior to the *in vivo* administration of the resulting recombinant cell by any method known within the art including, but not limited to: transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences of interest, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, and similar methodologies which ensure that the necessary developmental and physiological functions of the recipient cells are not disrupted by the transfer. See *e.g.*, Loeffler & Behr, 1993. *Meth. Enzymol.* 217: 599-618. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

In preferred embodiments of the present invention, the resulting recombinant cells may be delivered to a patient by various methods known within the art including, but not limited to: injection of epithelial cells (*e.g.*, subcutaneously); the application of recombinant skin cells as a skin graft onto the patient and the intravenous injection of recombinant blood cells (*e.g.*, hematopoetic stem or progenitor cells). The total amount of cells which are envisioned for use depend upon the desired effect, patient state, etc., and may be determined by one skilled within the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes and blood cells (*e.g.*, T-lymphocytes,

B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes and hematopoetic stem or progenitor cells obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.). In a preferred embodiment of the present invention, the cell utilized for gene therapy may be autologous to the patient.

In a specific embodiment in which recombinant cells are used in gene therapy, stem or progenitor cells, which can be isolated and maintained *in vitro*, may be utilized. Such stem cells include, but are not limited to, hematopoetic stem cells (HSC), stem cells of epithelial tissues (*e.g.*, skin, lining of the gut, embryonic heart muscle cells, liver stem cells) and neural stem cells (see *e.g.*, Stemple & Anderson, 1992. *Cell* 71:973-985). With respect to hematopoetic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSC may be used in this specific embodiment of the invention. As previously discussed, the HSCs utilized for gene therapy are, preferably, autologous to the patient. Hence, non-autologous HSCs are, preferably, utilized in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. See *e.g.*, Kodo, *et al.*, 1984. *J. Clin. Invest.* 73:1377-1384. In another preferred embodiment of the present invention, HSCs may be highly enriched (or produced in a substantially-pure form), by any techniques known within the art, prior to administration to the patient. See *e.g.*, Witlock & Witte, 1982. *Proc. Natl. Acad. Sci. USA* 79:3608-3612.

(8) Utilization of Anti-Sense Oligonucleotides

In a specific embodiment of the present invention, CDK2 protein•CDK2 protein-IP complex formation and function may be inhibited by the use of anti-sense nucleic acids for the CDK2 protein and/or a CDK2 protein-IP (*e.g.*, cyclin I, ERH, hsReq*-1 and hsReq*-2), and is preferably comprised of both the CDK2 protein and the CDK2 protein-IP. In addition, the present invention discloses the therapeutic or prophylactic use of nucleic acids (of at least six nucleotides in length) which are anti-sense to a genomic sequence (gene) or cDNA encoding the CDK2 protein and/or a CDK2 protein-IP, or portions thereof. Such anti-sense nucleic acids have utility as Therapeutics which inhibit CDK2 protein•CDK2 protein-IP complex formation or activity, and may be utilized in a therapeutic or prophylactic manner.

Another specific embodiment of the present invention discloses methodologies for the inhibition of the expression of the CDK2 protein and a CDK2 protein-IP nucleic acid sequences,

within a prokaryotic or eukaryotic cell, which is comprised of providing the cell with an therapeutically-effective amount of an anti-sense nucleic acid of the CDK2 protein and a CDK2 protein-IP, or derivatives thereof.

The anti-sense nucleic acids of the present invention may be oligonucleotides which may either be directly administered to a cell or which may be produced *in vivo* by transcription of the exogenous, introduced sequences. In addition, the anti-sense nucleic acid may be complementary to either a coding (*i.e.*, exonic) and/or non-coding (*i.e.*, intronic) region of the CDK2 protein or CDK2 protein-IP mRNAs. The CDK2 protein and CDK2 protein-IP anti-sense nucleic acids are, at least, six nucleotides in length and are, preferably, oligonucleotides ranging from 6-200 nucleotides in length. In specific embodiments, the anti-sense oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The anti-sense oligonucleotides may be DNA or RNA (or chimeric mixtures, derivatives or modified versions thereof), may be either single-stranded or double-stranded and may be modified at a base, sugar or phosphate backbone moiety.

In addition, the anti-sense oligonucleotide of the present invention may include other associated functional groups, such as peptides, moieties which facilitate the transport of the oligonucleotide across the cell membrane, a hybridization-triggered cross-linking agent, a hybridization-triggered cleavage-agent, and the like. See *e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; PCT Publication No. WO 88/09810. In a specific embodiment, the CDK2 protein and CDK2 protein-IP antisense oligonucleotides comprise catalytic RNAs or ribozymes. See, *e.g.*, Sarver, *et al.*, 1990. *Science* 247:1222-1225.

The anti-sense oligonucleotides of the present invention may be synthesized by standard methodologies known within the art including, but not limited to: (*i*) automated phosphorothioate-mediated oligonucleotide synthesis (see *e.g.*, Stein, *et al.*, 1988. *Nuc. Acids Res.* 16:3209) or (*ii*) methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (see *e.g.*, Sarin, *et al.*, 1988. *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451).

In an alternative embodiment, the CDK2 protein and CDK2 protein-IP antisense nucleic acids are produced intracellularly by transcription of an exogenous sequence. For example, a vector may be produced which (upon being exocytosed by the cell) is transcribed *in vivo*, thus producing an antisense nucleic acid (RNA) species. The aforementioned vector may either

remain episomal or become chromosomally-integrated, so long as it can be transcribed to produce the desired antisense RNA. The vectors utilized in the practice of the present invention may be derived from bacterial, viral, yeast or other sources known within the art, which are utilized for replication and expression in mammalian cells. Expression of the sequences encoding the CDK2 protein and CDK2 protein-IP antisense RNAs may be facilitated by any promoter known within the art to function in mammalian, preferably, human cells. Such promoters may be inducible or constitutive and include, but are not limited to: (*i*) the SV40 early promoter region; (*ii*) the promoter contained in the 3'-terminus long terminal repeat of Rous sarcoma virus (RSV); (*iii*) the Herpesvirus thymidine kinase promoter and (*iv*) the regulatory sequences of the metallothionein gene.

The CDK2 protein and CDK2 protein-IP antisense nucleic acids may be utilized prophylactically or therapeutically in the treatment or prevention of disorders of a cell type which expresses (or preferably over-expresses) the CDK2 protein•CDK2 protein-IP complex. Cell types which express or over-express the CDK2 protein and CDK2 protein-IP RNA, or hsReq*-1 and hsReq*-2 RNA, may be identified by various methods known within the art including, but are not limited to, hybridization with CDK2 protein- and CDK2 protein-IP-specific nucleic acids (*e.g.*, by Northern hybridization, dot blot hybridization, *in situ* hybridization) or by observing the ability of RNA from the specific cell type to be translated *in vitro* into the CDK2 protein and the CDK2 protein-IP by immunohistochemistry. In a preferred aspect, primary tissue from a patient may be assayed for the CDK2 protein and/or CDK2 protein-IP expression prior to actual treatment by, for example, immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the present invention, comprising an effective amount of a CDK2 protein and a CDK2 protein-IP antisense nucleic acid contained within a pharmaceutically-acceptable carrier may be administered to a patient having a disease or disorder which is of a type that expresses or over-expresses CDK2 protein•CDK2 protein-IP complex RNA or protein. The amount of CDK2 protein and/or CDK2 protein-IP antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will be dependant upon the nature of the disorder or condition, and may be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity *in vitro*, and then in useful animal model systems prior to testing and use in humans. In a specific embodiment, pharmaceutical compositions comprising CDK2 protein and CDK2 protein-IP

antisense nucleic acids may be administered via liposomes, microparticles, or microcapsules. See *e.g.*, Leonetti, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87:2448-2451.

(9) CDK2 Protein•CDK2 Protein-IP Complex Assays

The functional activity of CDK2 protein•CDK2 protein-IP complexes (and derivatives, fragments, analogs and homologs thereof) may be assayed by a number of methods known within the art. For example, putative modulators (*e.g.*, inhibitors, agonists and antagonists) of CDK2 protein•CDK2 protein complex activity (*e.g.*, anti-CDK2 protein•CDK2 protein-IP complex antibodies, as well as CDK2 protein or CDK2 protein-IP antisense nucleic acids) may be assayed for their ability to modulate CDK2 protein•CDK2 protein-IP complex formation and/or activity.

(*i*) Immunoassays

In a specific embodiment of the present invention, immunoassay-based methodologies are disclosed where one is assaying for: (*i*) the ability to bind to, or compete with, wild-type CDK2 protein•CDK2 protein-IP complex or hsReq*-1 or hsReq*-2or (*ii*) the ability to bind to an anti-CDK2 protein•CDK2 protein-IP complex antibody. These immunoassays include, but are not limited to, competitive and non-competitive assay systems utilizing techniques such as radioimmunoassays, enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels), Western blots, Northwestern blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein-A assays and immunoelectrophoresis assays, and the like. In one specific embodiment of the present invention, antibody binding is detected by assaying for a label on the primary antibody. In another specific embodiment, the binding of the primary antibody is ascertained by the detection of the binding of a secondary antibody (or reagent) specific for the primary antibody. In a further embodiment, the secondary antibody is labeled.

(*ii*) Gene Expression Assays

The expression of the CDK2 protein or CDK2 protein-IP genes (both endogenous genes and those expressed from recombinant DNA) may be detected using techniques known within the art including, but not limited to: Southern hybridization, Northern hybridization, restriction endonuclease mapping, DNA sequence analysis and polymerase chain reaction amplification (PCR) followed by Southern hybridization or RNase protection (see *e.g., Current Protocols in Molecular Biology* 1997. (John Wiley and Sons, New York, NY)) with probes specific for the CDK2 protein and CDK2 protein-IP genes in various cell types.

In one specific embodiment of the present invention, Southern hybridization may be used to detect genetic linkage of the CDK2 protein and/or CDK2 protein-IP gene mutations to physiological or pathological states. Numerous cell types, at various stages of development, may be characterized for their expression of the CDK2 protein and a CDK2 protein-IP (particularly the concomitant expression of the CDK2 protein and CDK2 protein-IP within the same cells). The stringency of the hybridization conditions for Northern or Southern blot analysis may be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific probes used. Modification of these aforementioned methods, as well as other methods well-known within the art, may be utilized in the practice of the present invention.

(*iii*) Binding Assays

Derivatives, fragments, analogs and homologs of CDK2 protein-IPs may be assayed for binding to the CDK2 protein by any method known within the art including, but not limited to: (*i*) the modified yeast two hybrid assay system; (*ii*) immunoprecipitation with an antibody which binds to the CDK2 protein within a complex, followed by analysis by size fractionation of the immunoprecipitated proteins (*e.g.*, by denaturing or non-denaturing polyacrylamide gel electrophoresis); (*iii*) Western analysis; (*v*) non-denaturing gel electrophoresis, and the like.

(*iv*) Assays for Biological Activity

A specific embodiment of the present invention provides a methodology for the screening of a derivative, fragment, analog or homolog of the CDK2 protein for biological activity which is comprised of contacting a derivative, fragment, analog or homolog of the CDK2 protein with one of the CDK2 Protein-IPs (*e.g.*, cyclin I, ERH, hsReq*-1 and hsReq*-2) and detecting the

formation of a complex between said derivative, fragment, analog or homolog of the CDK2 protein and the specific CDK2 protein-IP; wherein the detection of the formation of said complex indicates that the CDK2 protein derivative, fragment, analog or homolog, possesses biological (*e.g.*, binding) activity. Similarly, an additional embodiment discloses a methodology for the screening a derivative, fragment, analog or homolog of a CDK2 protein-IP for biological activity comprising contacting said derivative, fragment, analog or homolog of said protein with the CDK2 protein; and detecting the formation of a complex between said derivative, fragment, analog or homolog of the CDK2 protein-IP and the CDK2 protein; wherein detecting the formation of said complex indicates that said the CDK2 protein-IP derivative, fragment, analog, or homolog possesses biological activity.

(10) Modulation of CDK2 Protein•CDK2 Protein-IP Complex Activity

The present invention discloses methodologies relating to the modulation of the activity of a protein moiety which possesses the ability to participate in a CDK2 protein•CDK2 protein-IP complex (*e.g.*, the CDK2 protein, cyclin I, ERH, hsReq*-1 and hsReq*-2) by the administration of a binding partner of that protein (or derivative, fragment, analog or homolog thereof). The CDK2 protein (and derivatives, fragments, analogs and homologs thereof) may be assayed for their ability to modulate the activity or levels of a CDK2 protein-IP by contacting a cell, or administering to an animal expressing a CDK2 protein-IP gene, with the CDK2 protein, or a nucleic acid encoding the CDK2 protein or an antibody which immunospecifically-binds the CDK2 protein, or a derivative, fragment, analog or homolog of said antibody which contains the binding domain thereof, and measuring a change in CDK2 protein-IP levels or activity; wherein a change in CDK2 protein-IP levels or activity indicates that the CDK2 protein possesses the ability to modulate CDK2 protein-IP levels or activity. In another embodiment, a CDK2 protein-IP may be assayed for the ability to modulate the activity or levels of the CDK2 protein in an analogous manner.

(11) CDK2-Related Treatment Assays

(*i*) Tumorigenesis

The CDK2 protein, and several of the identified binding partners of the CDK2 protein (*i.e.*, CDK2 protein-IPs) have roles in the control of cell proliferation and, therefore, cell-transformation and tumorigenesis. Accordingly, the present invention discloses methodologies for screening CDK2 protein•CDK2 protein-IP complexes and CDK2 protein-IPs (and derivatives, fragments, analogs and homologs, thereof) for the ability to alter cell proliferation, cell transformation and/or tumorigenesis *in vitro* and *in vivo*. For example, but not by way of limitation, cell proliferation may be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, c-*fos*, c-*myc*) cell-cycle markers, and the like.

The CDK2 protein•CDK2 protein-IP complexes and CDK2 protein-IPs (and derivatives, fragments, analogs and homologs, thereof) may also be screened for activity in inducing or inhibiting cell transformation (or the progression to malignant phenotype) *in vitro*. The proteins and protein complexes of the present invention may be screened by contacting either cells with a normal phenotype (for assaying for cell transformation) or a transformed cell phenotype (for assaying for inhibition of cell transformation) with the protein or protein complex of the present invention and examining the cells for acquisition or loss of characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) including, but not limited to: colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250 Kdal cell-surface protein, and the like. See *e.g.*, Luria, *et al.*, 1978. *General Virology, 3rd ed.* (John Wiley & Sons, New York, NY).

The CDK2 protein•CDK2 protein-IP complexes (and derivatives, fragments, analogs and homologs, thereof) may also be screened for activity to promote or inhibit tumor formation *in vivo* in non-human test animal. A vast number of animal models of hyperproliferative disorders (*e.g.*, tumorigenesis and metastatic spread) are known within the art. See *e.g.*, Lovejoy, *et al.*, 1997. *J. Pathol.* 181:130-135. In a specific embodiment of the present invention, the proteins

and protein complexes may be administered to a non-human test animal (preferably a test animal predisposed to develop a type of tumor) and the non-human test animals is subsequently examined for an increased incidence of tumor formation in comparison with controls animals which were not administered the proteins or protein complex of the present invention. Alternatively, the proteins and protein complexes may be administered to non-human test animals possessing tumors (*e.g.*, animals in which tumors have been induced by introduction of malignant, neoplastic, or transformed cells or by administration of a carcinogen) and subsequently examining the tumors within the test animals for tumor regression in comparison to controls. Accordingly, once a hyperproliferative disease or disorder has been shown to be amenable to treatment by modulation of CDK2 protein•CDK2 protein-IP complex activity that disease or disorder may be treated or prevented by administration of a Therapeutic which modulates CDK2 protein•CDK2 protein-IP complex formation.

(*ii*) Atherosclerosis

The CDK2 protein plays a role in the regulation of atherosclerosis. Accordingly, the present invention discloses methodologies for screening CDK2 protein•CDK2 protein-IP complexes and CDK2 protein-IPs (and derivatives, fragments, analogs and homologs, thereof) for the ability to alter atherosclerosis *in vitro* and *in vivo*.

A vast array of animal and cell culture models exist for processes involved in atherosclerosis. A limited and non-exclusive list of animal models includes knockout mice for premature atherosclerosis (see *e.g.*, Kurabayashi & Yazaki, 1996. *Int. Angiol.* 15:187-194); transgenic mouse models of atherosclerosis (see *e.g.*, Kappel, *et al.*, 1994. *FASEB J.* 8:583-592); antisense oligonucleotide treatment of animal models (see *e.g.*, Callow, 1995. *Curr. Opin. Cardiol.* 10:569-576) and transgenic rabbit models for atherosclerosis (see *e.g.*, Taylor, 1997. *Ann. N.Y. Acad. Sci.* 811:146-152).

In addition, *in vitro* cell models include but are not limited to monocytes exposed to low density lipoprotein (see *e.g.*, Frostegard, *et al.*, 1996. *Atherosclerosis* 121:93-103); cultured human aortic endothelial cells (see *e.g.*, Farber, *et al.*, 1992. *Am. J. Physiol.* 262:1088-1085) and foam cell cultures (see *e.g.*, Libby, *et al.*, 1996. *Curr Opin Lipidol.* 7:330-335). Potentially effective Therapeutics, for example but not by way of limitation, reduce foam cell formation in

cell culture models, or reduce atherosclerotic plaque formation in hypercholesterolemic mouse models of atherosclerosis.

(12) Protein-Protein Interaction Assays

The present invention discloses methodologies for assaying and screening derivatives, fragments, analogs and homologs of CDK2 protein-interacting proteins (CDK2 protein-IPs) for binding to CDK2 protein. The derivatives, fragments, analogs and homologs of the CDK2 protein-IPs which interact with CDK2 protein may be identified by means of a yeast two hybrid assay system (see *e.g.*, Fields & Song, 1989. *Nature* 340:245-246) or; preferably, a modification and improvement thereof, as described in U.S. Patent Applications Serial Nos. 08/663,824 (filed June 14, 1996) and 08/874,825 (filed June 13, 1997), both of which are entitled "Identification and Comparison of Protein-Protein Interactions that Occur in Populations and Identification of Inhibitors of These Interactions," to Nandabalan, *et al.*, and which are incorporated by reference herein in their entireties.

The identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of the expression of a reporter gene (hereinafter "Reporter Gene"), the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The bait CDK2 protein (or derivative, fragment, analog or homolog) and prey protein (proteins to be tested for ability to interact with the bait protein) are expressed as fusion proteins to a DNA-binding domain, and to a transcriptional regulatory domain, respectively, or *vice versa*. In a specific embodiment of the present invention, the prey population may be one or more nucleic acids encoding mutants of a CDK2 protein-IP (*e.g.*, as generated by site-directed mutagenesis or another method of producing mutations in a nucleotide sequence). Preferably, the prey populations are proteins encoded by DNA (*e.g.*, cDNA, genomic DNA or synthetically generated DNA). For example, the populations may be expressed from chimeric genes comprising cDNA sequences derived from a non-characterized sample of a population of cDNA from mammalian RNA. In another specific embodiment, recombinant biological libraries expressing random peptides may be used as the source of prey nucleic acids.

The present invention discloses methods for the screening for inhibitors of the interacting proteins (CDK2 protein-IPs). In brief, the protein-protein interaction assay may be performed as

previously described herein. with the exception that it is performed in the presence of one or more candidate molecules. A resulting increase or decrease in Reporter Gene activity, in relation to that which was present when the one or more candidate molecules are absent. indicates that the candidate molecule exerts an effect on the interacting pair. In a preferred embodiment, inhibition of the protein interaction is necessary for the yeast cells to survive. for example, where a non-attenuated protein interaction causes the activation of the *URA3* gene. causing yeast to die in medium containing the chemical 5-fluoroorotic acid. See *e.g.*, Rothstein. 1983. *Meth. Enzymol.* 101:167-180.

In general. the proteins comprising the bait and prey populations are provided as fusion (chimeric) proteins, preferably by recombinant expression of a chimeric coding sequence containing each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA-binding domain which may be any DNA-binding domain, so long as it specifically recognizes a DNA sequence within a promoter (*e.g.*, a transcriptional activator or inhibitor). For the other population, the pre-selected sequence is an activator or inhibitor domain of a transcriptional activator or inhibitor, respectively. The regulatory domain alone (not as a fusion to a protein sequence) and the DNA-binding domain alone (not as a fusion to a protein sequence) preferably, do not detectably interact, so as to avoid false-positives in the assay. The assay system further includes a reporter gene operably linked to a promoter which contains a binding site for the DNA-binding domain of the transcriptional activator (or inhibitor). Accordingly, in the practice of the present invention. the binding of the CDK2 protein fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or inhibitor), which concomitantly activates (or inhibits) expression of the Reporter Gene.

In a specific embodiment, the present invention discloses a methodology for detecting one or more protein-protein interactions comprising the following steps: (*i*) recombinantly-expressing the CDK2 protein (or a derivative, fragment. analog or homolog thereof) in a first population of yeast cells of a first mating type and possessing a first fusion protein containing the CDK2 protein sequence and a DNA-binding domain; wherein said first population of yeast cells contains a first nucleotide sequence operably-linked to a promoter which is "driven" by one or more DNA-binding sites recognized by said DNA-binding domain such that an interaction of said first fusion protein with a second fusion protein (comprising a transcriptional activation domain) results in increased transcription of said first nucleotide sequence; (*ii*) negatively

selecting to eliminate those yeast cells in said first population in which said increased transcription of said first nucleotide sequence occurs in the absence of said second fusion protein; (*iii*) recombinantly expressing in a second population of yeast cells of a second mating type different from said first mating type, a plurality of said second fusion proteins; wherein said second fusion protein is comprised of a sequence of a derivative, fragment, analog or homolog of a CDK2 protein-IP and an activation domain of a transcriptional activator, in which the activation domain is the same in each said second fusion protein; (*iv*) mating said first population of yeast cells with said second population of yeast cells to form a third population of diploid yeast cells, wherein said third population of diploid yeast cells contains a second nucleotide sequence operably linked to a promoter "driven" by a DNA-binding site recognized by said DNA-binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of said second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different and (*v*) detecting said increased transcription of said first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

In a preferred embodiment, the bait (a CDK2 protein sequence) and the prey (a library of chimeric genes) are combined by mating the two yeast strains on solid media for a period of approximately 6-8 hours. In a less preferred embodiment, the mating is performed in liquid media. The resulting diploids contain both types of chimeric genes (*i.e.*, the DNA-binding domain fusion and the activation domain fusion). After an interactive population is obtained, the DNA sequences encoding the pairs of interactive proteins are isolated by a method wherein either the DNA-binding domain hybrids or the activation domain hybrids are amplified, in separate reactions. Preferably, the amplification is carried out by polymerase chain reaction (PCR; see *e.g.*, Innis, *et al.*, 1990. *PCR Protocols* (Academic Press, Inc., San Diego, CA)) utilizing pairs of oligonucleotide primers specific for either the DNA-binding domain hybrids or the activation domain hybrids. The PCR amplification reaction may also be performed on pooled cells expressing interacting protein pairs, preferably pooled arrays of interactants. Other amplification methods known within the art may also be used including, but not limited to, ligase chain reaction; Qβ-replicase or the like. See *e.g.*, Kricka, *et al.*, 1995. *Molecular Probing, Blotting, and Sequencing* (Academic Press, New York, NY).

In an additional embodiment of the present invention, the plasmids encoding the DNA-binding domain hybrid and the activation domain hybrid proteins may also be isolated and cloned by any of the methods well-known within the art. For example, but not by way of limitation, if a shuttle (yeast to *E. coli*) vector is used to express the fusion proteins, the genes may be subsequently recovered by transforming the yeast DNA into *E. coli* and recovering the plasmids from the bacteria. See *e.g.*, Hoffman *.et al.*, 1987. *Gene* 57:267-272.

(13) Pharmaceutical Compositions

The invention present discloses methods of treatment and prophylaxis by the administration to a subject of an pharmaceutically-effective amount of a Therapeutic of the invention. In a preferred embodiment, the Therapeutic is substantially purified and the subject is a mammal, and most preferably, human.

Formulations and methods of administration that can be employed when the Therapeutic comprises a nucleic acid are described in Sections 6(*i*) and 6(*ii*), *supra*. Various delivery systems are known and can be used to administer a Therapeutic of the present invention including, but not limited to: (*i*) encapsulation in liposomes, microparticles, microcapsules; (*ii*) recombinant cells capable of expressing the Therapeutic; (*iii*) receptor-mediated endocytosis (see, *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262:4429-4432); (*iv*) construction of a Therapeutic nucleic acid as part of a retroviral or other vector, and the like.

Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The Therapeutics of the present invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically-active agents. Administration can be systemic or local. In addition, it may be advantageous to administer the Therapeutic into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter attached to a reservoir (*e.g.*, an Ommaya reservoir). Pulmonary administration may also be employed by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. It may also be desirable to administer the Therapeutic locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local

infusion during surgery, topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant. In a specific embodiment, administration may be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment of the present invention, the Therapeutic may be delivered in a vesicle, in particular a liposome. See *e.g.*, Langer, 1990. *Science* 249:1527-1533. In yet another embodiment, the Therapeutic can be delivered in a controlled release system including ,but not limited to: a delivery pump (see *e.g.*, Saudek, *et al.*, 1989. *New Engl. J. Med.* 321:574 and a semi-permeable polymeric material (see *e.g.*, Howard, *et al.*, 1989. *J. Neurosurg.* 71:105). Additionally, the controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the brain), thus requiring only a fraction of the systemic dose. See, *e.g.*, Goodson, In: *Medical Applications of Controlled Release* 1984. (CRC Press, Boca Raton, FL).

In a specific embodiment of the present invention, where the Therapeutic is a nucleic acid encoding a protein, the Therapeutic nucleic acid may be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular (*e.g.*, by use of a retroviral vector, by direct injection, by use of microparticle bombardment, by coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:1864-1868), and the like. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically-effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. As utilized herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered and includes, but is not limited to such sterile liquids as water and oils.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and may

be determined by standard clinical techniques by those of average skill within the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the overall seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration of the Therapeutics of the present invention are generally about 20-500 micrograms (μg) of active compound per kilogram (kg) body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The present invention also provides a pharmaceutical pack or kit, comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions and Therapeutics of the present invention. Optionally associated with such container(s) may be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

(14) Specific Examples

(*i*) Identification of CDK2 Protein•CDK2 Protein-IP Complexes

A modified, improved yeast two hybrid system was used to identify protein interactions of the present invention. Yeast is an eukaryote, and therefore any intermolecular protein interactions detected in this type of system demonstrate protein interactions that occur under physiological conditions. See *e.g.*, Chien, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88:9578-9581. Expression vectors were constructed to encode two hybrid proteins. For a "forward" screen, one hybrid consisted of the DNA binding domain of the yeast transcriptional activator Gal4 fused to a portion of CDK2. The other hybrid consisted of the Gal4 activator domain fused to "prey" protein sequences encoded by a mammalian cDNA library. Each of the resulting vectors was then inserted into complementary mating types of yeast (an a mating type and an α mating type) by use of techniques well-known within the art. See *e.g.*, Chien, *et al.*, 1991, *supra*. Mating was

carried out to express both vector constructs within the same yeast cells. thus allowing protein-protein interaction to occur. Interaction between the bait and prey domains led to transcriptional activation of Reporter Genes containing *cis*-binding elements for Gal4. The Reporter Genes encoding the indicator protein β-galactosidase, and metabolic markers for uracil and histidine auxotrophy, were included in a specific fashion, in one or the other of the yeast strains utilized in the mating. In this manner, yeast were selected for successful mating, expression of both fusion constructs, *i.e.*, CDK2 and CDK2-IP fusion proteins. Yeast clones which were found to contain interacting regions were selected and grown in individual wells of 96-well microtiter plates. The plasmids containing the CDK2 protein-IP sequences were then isolated and characterized.

The prey cDNAs were obtained from a fetal brain cDNA library of 3.5×10^6 independent isolates (Clontech, Palo Alto, CA). The library was synthesized from *Xho 1*-digested and dT15-primed fetal brain mRNA (derived from five male/female, 19-22 week fetuses) which was directionally cloned into pACT2 (Clontech; Palo Alto, CA), a yeast Gal4 activation domain cloning vector including the *LEU2* gene for selection of yeast deficient in leucine biosynthesis.

Screens were performed in order to test the interaction of prey cDNA products against an array of bait proteins. The bait was encoded by the CDK2 protein nucleotide sequence comprised of nucleotides 1-897 (amino acids 1-298), as depicted in Figure 1 [SEQ ID NO:1] and [SEQ ID NO:2], respectively.

The nucleic acid encoding the introduced bait was then expressed by lithium acetate-polyethylene glycol-mediated transformation (see *e.g.*, Ito, *et al.*, 1983. *J. Bacteriol.* 153:163-168) into the yeast strain YULH (mating type a, *ura3*, *his3*, *lys2*, *Ade2*, *trp1*, *leu2*, *gal4*, *gal80*, *GAL1-URA3*, *GAL1-lacZ*); whereas the prey sequences were introduced by transformation into the yeast strain N106r (mating type á, *ura3*, *his3*, *ade2*, *trp1*, *leu2*, *gal4*, *gal80*, *cyh*^{r}, *Lys2::GAL1*$_{UAS}$*-HIS3*$_{TATA}$*-HIS3*, *ura3::GAL1*$_{UAS}$*-GAL*$_{TATA}$*-lacZ*). The transformed yeast populations were then mated using standard methods in the art. See *e.g.*, Sherman, *et al.*, 1991. *Getting Started with Yeast* (Academic Press; New York, NY). In brief, the yeast cells were grown until mid- to late-log phase on media that selected for the presence of the appropriate plasmids. The two mating strains (α and a) were then diluted in YAPD media, filtered onto nitrocellulose membranes, incubated at 30°C for 6-8 hours and then transferred to media selective for the desired diploids (*i.e.*, yeast harboring Reporter Genes for β-galactosidase, uracil auxotrophy, and histidine auxotrophy and expression of the vectors encoding the bait and prey). The mating

products were then plated onto synthetic complete (SC) media (see *e.g.*, Kaiser, *et al.*, 1994. *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY)) lacking adenine and lysine (to facilitate the selection of successful matings), leucine and tryptophan (to facilitate the selection for expression of genes encoded by both the bait and prey plasmids) and uracil and histidine (to facilitate the selection for protein interactions). This medium containing the aforementioned compounds is referred to as SC Selective medium (hereinafter "SCS medium").

Selected clones were examined for expression of β-galactosidase to confirm the formation of a CDK2 protein•CDK2 protein-IP interaction. Filter-lift β-galactosidase assays were then performed as per a modified of the protocol of Breeden & Nasmyth (1985. *Cold Spring Harbor Quant. Biol.* 50: 643-650). Colonies were patched onto SCS plates, grown overnight and replica-plated onto Whatman No. 1 filters. The replica filters were subsequently assayed for β-galactosidase activity (*i.e.*, colonies which were positive turned a visible blue).

The cells contained within colonies which were positive for protein interaction contained a mixture of DNA-binding and activation-domain plasmids and these cells were individually plated and regrown as single isolates in the individual wells of 96-well microtiter plates. Ten microliters (μl) of each isolate was lysed, the inserts were amplified by PCR using primers specific for the flanking sequences of each vector and approximately 200 amino-terminal nucleotides of each insert sequence was determined using an ABI Model 377 sequenator. Comparison to known sequences was made using the "BLAST" computer program publicly available through the National Center for Biotechnology Information.

During a subsequent screening procedure utilizing a fragment of the CDK2 protein, identified sequences included one isolate identical to the *cyclin I* sequence starting at nucleotide 46 (as depicted in Figure 2 (SEQ ID NO:3)), one isolate identical to the *ERH* sequence starting from nucleotide 153 (as depicted in Figure 3 (SEQ ID NO:5)), and two isolates identical to the *hsReq* sequence starting from nucleotides 1789 and 1819 (as depicted in Figure 4 (SEQ ID NO:7)). The determined nucleic acid sequences and corresponding amino acid sequences of cyclin I, ERH, and splice variants hsReq*-1 and hsReq*-2 are shown in Figures 2-4, 6, and 7, respectively. A summary of the CDK2 and CDK2-IP interacting domains is shown in Figure 7.

(*ii*) Verification of the Specificity of the CDK2 Protein-Interactions

To determine the overall degree of specificity for the bait:prey interaction, two general assays were performed. In the first assay, N106r yeast cells were produced which expressed the individual plasmids encoding the CDK2 proteins. These yeast cells were plated on SCS plates, grown overnight, and examined for growth. No growth was found for all five interactants, thus confirming that they were not "self-activating" proteins (*i.e.*, these proteins require interaction with a second protein domain for a functional activation complex).

In the second assay, plasmids containing cyclin I, ERH, hsReq*-1 and hsReq*-2 inserts were transformed into strain N106r (mating type α) and mated with yeast strain YULH yeast (mating type a) expressing proteins other than the CDK2 protein. Promiscuous binders (*i.e.*, inserts able to bind with many other proteins in a non-specific manner) would interact in a non-specific manner with non-CDK2 protein domains, and would subsequently be discarded as non-specific interactants. It should be noted that none of the interactants of the present invention showed binding to protein other than those described in the following paragraph.

In order to recapitulate the aforementioned detected interactions, and further demonstrate their specificity, the isolated bait plasmid for the CDK2 protein, along with the plasmid encoding human bait protein 1 (B1), was used to transform yeast YULH (mating type a). The interacting domains from cyclin I, ERH, hsReq*-1 and hsReq*-2 were transformed into strain). N106r (mating type α). The transformants were re-amplified and a mating was performed to recapitulate the identified CDK2 protein•CDK2 protein-IP interactions. As shown in Figure 8, CDK2 complexed specifically with ERH (Box B), and hsReq*-1 and/or hsReq*-2 (Box E), as well as the known interactants cyclin H (Box A), p27 (Box C) and p21 (Box D). It did not react non-specifically with the prey P1. As illustrated in Figure 8, the intersection of the CDK2 row (top) with the ERH, p21, p27, and hsReq*-1 and/or hsReq*-2 columns indicates growth (*i.e.* a positive interaction), but the intersection of the CDK2 row with the column for P1 indicates no growth, *i.e.*, no protein interaction.

(*iii*) Analysis of the Sequences Encoding hsReq*-1 and hsReq*-2

Regions within the 3' untranslated regions of the known protein cDNAs for hsReq were identified as encoding a protein or proteins that interact with CDK2 using the modified yeast two hybrid system. The present invention discloses interacting nucleic acid sequences

identical to the nucleotide sequence of hsReq from nucleotide base 1788 to the end and from 1818 to the end (as depicted in Figure 4 (SEQ ID NO:7)).

These regions did not encode open reading frames (ORFs) sufficient to encode a protein. This was determined by performing a "BLAST" analysis to determine translations in the three possible forward reading frames. Within the detected regions, no ORF of 60 amino acids or greater, beginning with an initiator methionine, and no ORF beginning from the 5' end that could represent the carboxyl-terminus of a protein of 60 amino acids or longer, was detected for any of the three detected inserts. Thus, the sequences were examined to determine if they could encode splice variants of the known hsReq protein that included the detected interacting sequences.

Determination of 5' and 3' splice points for protein splice variants was performed as described *supra*. Potential 3' intron:exon splice sites were identified based on the consensus analysis described by Padgett *et al.*, 1984 (Ann. Rev. Biochem. 55:1119-1150) and *supra*. Based on the known translational frame of the mature protein and each predicted 5' splice site, compatible translational frames for successful splicing were defined for potential 3' splice sites. Nucleic acid sequences were analyzed by a number of nucleotide sequence analysis programs available in the art to define possible protein translation products. Translation in the three forward translation frames was used to define possible open reading frames (contiguous spans of codons for amino acids without the presence of a stop codon). Only 3' sites that matched the necessary translational frame of a 5'-splice junction were retained. Unmatched 5'- or 3'-splice sites were eliminated. Thus, sites containing three non-C, non-T bases upstream of the splice site were included, resulting in two possible 3'-splice sites for *hsReq* (for the splice variants *hsReq*-1* and *hsReq*-2*, respectively).

Finally, for each possible 5':3' splice site pair, a search for a mammalian branch point consensus sequence (T/C N CTGAC) was performed (see *e.g.*, Reed & Maniatis, 1988. *Genes Dev.* 2:1268-1276). Each splice variant for *hsReq* (*i.e.*, *hsReq*-1* and *hsReq*-2*) had a branch point consensus sequence (Figure 4).

Splice variant proteins must encode at least 60 amino acid residues to constitute a viable *in vivo* product. Further, the 3' end of the splice variants must, by definition, extend into the identified interacting sequence. The splice sites for the splice variants *hsReq*-1* and *hsReq*-2* met these requirements. Specifically, for both *hsReq*-1* and *hsReq*-2*, a 5'splice site was identified at nucleotides 563-570 of the *hsReq* sequence as depicted in Figure 4 (SEQ ID NO:7).

with this 5' splice site indicated as B in Figure 4. For *hsReq*-1*, a 3' splice site was identified at nucleotides 1566 to 1580 and the branch point consensus sequence at nucleotides 1553 to 1544 of the *hsReq* nucleotide sequence (as depicted in Figure 4) indicated in Figure 4 as "E" and "D", respectively. For *hsReq*-2*, an alternative 3' splice site was identified at nucleotides 1776 to 1790 and the related branch point consensus sequence at nucleotides 1759-1765 of the *hsReq* nucleotide sequence (as depicted in Figure 4 (SEQ ID NO:7)), indicated in Figure 4 as "G" and "F", respectively.

Splice variant sequences were subjected to a further searches of the NRDB, a non-redundant compilation of GenBank CDS translations+PDB+SwissProt+PIR SwissProt sequences, to detect homologies to known protein sequences that were not detected over the span of the known protein sequences. No significant homologies to known proteins were detected for hsReq*-1 and hsReq*-2 utilizing this analysis.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention, in addition to those described herein, will become apparent to those skilled within the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, and the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

(1) A purified complex of a CDK2 protein and a CDK2 protein-IP protein, wherein said CDK2 protein-IP protein is selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2.

(2) The purified complex of claim 1, wherein said proteins are human proteins.

(3) A purified complex selected from the group consisting of a complex of a derivative of a CDK2 protein and a CDK2 protein-IP protein, a complex of a CDK2 protein and a derivative of a CDK2 protein-IP, and a complex of a derivative of a CDK2 protein and a derivative of a CDK2 protein-IP, in which the derivative of the CDK2 protein is capable of forming a complex with a wild-type CDK2 protein-IP protein and the derivative of the CDK2 protein-IP is capable of forming a complex with a wild-type CDK2 protein, wherein the CDK2 protein-IP protein is selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2.

(4) The purified complex of claim 3, wherein the derivative of the CDK2 protein and/or the CDK2 protein-IP protein is detectably-labeled with a label selected from a group consisting of: radioactive, fluorescent, chemiluminescent, colorimetric, or enzymatic moieties.

(5) A chimeric protein comprising a fragment of a CDK2 protein consisting of, at least, 6 amino acid residues joined via a covalent bond to a fragment of a CDK2 protein-IP protein also consisting of, at least, 6 amino acid residues.

(6) The chimeric protein of claim 5, wherein the fragment of the CDK2 protein is a fragment capable of binding a CDK2 protein-IP protein and in which the fragment of the CDK2 protein-IP protein is a fragment capable of binding the CDK2 protein.

(7) The chimeric protein of claim 6, wherein the fragment of the CDK2 protein and the fragment of the CDK2 protein-IP protein interact to form a CDK2 protein•CDK2 protein-IP complex.

(8) An antibody which immunospecifically-binds the complex of claim 1 or a fragment or derivative of said antibody containing the binding domain thereof.

(9) The antibody of claim 8, which does not immunospecifically bind a CDK2 protein or a CDK2 protein-IP protein which are not part of a CDK2 protein•CDK2 protein-IP complex.

(10) An isolated nucleic acid or an isolated plurality of nucleic acids comprising a nucleotide sequence encoding a CDK2 protein and a nucleotide sequence encoding a CDK2 protein-IP protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2.

(11) The isolated nucleic acid or isolated plurality of nucleic acids of claim 10 which are comprised of nucleic acid vectors.

(12) The isolated nucleic acid or isolated plurality of nucleic acids of claim 11, wherein the CDK2 protein coding sequence and the CDK2 protein-IP protein coding sequence are operably-linked to a promoter.

(13) An isolated nucleic acid which comprises a nucleotide sequence encoding the chimeric protein of claim 7.

(14) A cell containing a nucleic acid of claim 10, wherein the nucleic acid is a recombinant molecule.

(15) A cell containing a nucleic acid of claim 12, wherein the nucleic acid is a recombinant molecule.

(16) A recombinant cell containing a nucleic acid of claim 13, wherein the nucleic acid is a recombinant molecule.

(17) A purified protein selected from the group consisting of hsReq*-1 and hsReq*-2 proteins.

(18) The protein of claim 17, wherein said protein is a human protein.

(19) The protein of claim 18, which comprises an amino acid sequence selected from the group consisting of: SEQ ID NO.:11; and SEQ ID NO.:13.

(20) A purified protein encoded by a nucleic acid hybridizable to the inverse complement of DNA having a nucleotide sequence consisting of a portion of the nucleotide sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:10, said portion containing the splice site junction resulting from splicing of the unprocessed *hsReq* mRNA, said nucleic acid comprising a sequence absolutely complementary to an at least 10 nucleotide sequence spanning said splice site junction.

(21) A purified derivative or analog of the protein of claim 17, which derivative or analog can bind CDK2, which derivative or analog comprises at least a 10 amino acid portion of amino acids 187-280 of SEQ ID NO:9 or of amino acids 188-210 of SEQ ID NO:11.

(22) The derivative or analog of claim 21, which is capable of being bound by an antibody specific for a protein selected from the group consisting of: hsReq*-1 and hsReq*-2 proteins, which antibody does not bind hsReq.

(23) A purified fragment of the protein of claim 17, wherein said fragment are comprised of, at least, a 6 amino acid residue portion of said protein, the sequence of which protein is not contained in hsReq.

(24) A purified protein comprising an amino acid sequence which possesses at least a 60% identity to the protein of claim 17, wherein the percentage of identity is determined over an amino acid sequence of identical size to said protein of claim 17, and which protein is not hsReq.

(25) A chimeric protein comprising a fragment of the protein of claim 17, wherein said fragment consists of at least 6 amino acid residues of hsReq*-1 and hsReq*-2, which is joined via a covalent bond to an amino acid sequence of a second protein, wherein the second protein is not said protein of claim 17 and is not hsReq.

(26) An antibody which is capable of immunospecifically-binding the protein of claim 17, or a fragment or derivative of said antibody containing the binding domain thereof.

(27) An isolated nucleic acid comprising a nucleotide sequence encoding the protein of claim 18.

(28) An isolated nucleic acid comprising the nucleotide sequence of: SEQ ID NO.:10; and SEQ ID NO.:12.

(29) An isolated nucleic acid which is hybridizable to the inverse complement of a nucleic acid possessing a nucleotide sequence consisting of a portion of the nucleotide sequence of: SEQ ID NO.:10; and SEQ ID NO.:12, said portion containing the splice site junction resulting from splicing of the unprocessed hsReq mRNA and said nucleic acid comprising a sequence absolutely complementary to an at least 10 nucleotide sequence spanning said splice site junction.

(30) An isolated nucleic acid comprising a portion of the nucleotide sequence of SEQ ID NO.:10; and SEQ ID NO.:12, wherein said nucleic acid sequence is comprised of at least 10 nucleotides spanning the splice site junction resulting from splicing of the unprocessed *hsReq* mRNA.

(31) A cell containing the nucleic acid of claim 27, wherein said nucleic acid is a recombinant molecule.

(32) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the complex of claim 1 and a pharmaceutically-acceptable carrier.

(33) The pharmaceutical composition of claim 32, wherein said proteins are human proteins.

(34) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the complex of claim 3 and a pharmaceutically-acceptable carrier.

(35) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the chimeric protein of claim 5 and a pharmaceutically-acceptable carrier.

(36) A pharmaceutical composition of comprising a therapeutically- or prophylactically-effective amount of the chimeric protein of claim 6 and a pharmaceutically-acceptable carrier.

(37) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the antibody of claim 8, or a fragment or derivative of said antibody containing the binding domain thereof, and a pharmaceutically-acceptable carrier.

(38) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the antibody of claim 9, or a fragment or derivative of said antibody containing the binding domain thereof, and a pharmaceutically-acceptable carrier.

(39) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the nucleic acids or plurality of the nucleic acids of claim 10 and a pharmaceutically-acceptable carrier.

(40) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of isolated nucleic acid of claim 13 and a pharmaceutically-acceptable carrier.

(41) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the recombinant cell of claim 14 and a pharmaceutically-acceptable carrier.

(42) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the protein of claim 15 and a pharmaceutically-acceptable carrier.

(43) The pharmaceutical composition of claim 41, wherein the proteins comprise the amino acid sequence as set forth in SEQ ID NO.:11; and SEQ ID NO.:13.

(44) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of the antibody of claim 26, or a fragment or derivative of said antibody containing the binding domain thereof, and a pharmaceutically-acceptable carrier.

(45) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a nucleic acid comprising a nucleotide sequence encoding the protein of claim 17; and a pharmaceutically-acceptable carrier.

(46) A pharmaceutical composition comprising a therapeutically- or prophylactically-effective amount of a cell containing the recombinant nucleic acid of claim 27 and a pharmaceutically acceptable carrier.

(47) A methodology for the production of a complex of a CDK2 protein and a CDK2 protein-IP protein which is comprised of: (*i*) growing a recombinant cell containing the nucleic acid of claim 10 such that the encoded CDK2 protein and CDK2 protein-IP proteins are expressed and bind to each other and (*ii*) recovering the expressed complex of the CDK2 protein and the CDK2 protein-IP protein.

(48) A methodology for the production of a protein selected from the group consisting of: hsReq*-1 and hsReq*-2, which is comprised of: (*i*) growing a cell containing a recombinant nucleic acid encoding said protein such that the encoded protein is expressed and (*ii*) recovering the expressed protein.

(49) A methodology of diagnosing or screening for the presence of, or a predisposition for, developing a disease or disorder which is characterized by an aberrant level of a complex of a CDK2 protein and a CDK2 protein-IP protein, wherein the CDK2 protein-IP is selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, within a subject which is comprised of measuring the level of said complex, RNA encoding the CDK2 protein and CDK2 protein-IP

proteins or functional activity of said complex within a sample derived from the subject; wherein an increase or decrease in the level of said complex, said RNA encoding the CDK2 protein and CDK2 protein-IP protein, or functional activity of said complex within the sample, relative to the level of said complex, said RNA encoding the CDK2 protein and CDK2 protein-IP protein or functional activity of said complex found in an analogous sample derived from a subject not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

(50) A methodology of diagnosing or screening for the presence of, or a predisposition for, developing a disease or disorder which is characterized by an aberrant level of a protein or RNA selected from the group consisting of hsReq*-1 and hsReq*-2 protein or RNA, within a subject, which is comprised of measuring the level of said protein, said RNA or the functional activity of said protein within a sample derived from the subject; wherein an increase or decrease in the level of said protein, said RNA or said functional activity within the sample, relative to the level of said protein, said RNA or said functional activity found within an analogous sample derived from a subject not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

(51) A kit comprising, in one or more containers, a substance selected from the group consisting of a complex of a CDK2 protein and a CDK2 protein-IP, an antibody against said complex, nucleic acid probes which are capable of hybridizing to RNA encoding a CDK2 protein and RNA encoding a CDK2 protein-IP, or pairs of nucleic acid primers which are capable of priming the amplification of, at least, a portion of a gene encoding a CDK2 protein and gene encoding a CDK2 protein-IP, in which said CDK2 protein-IP is selected from the group consisting of: cyclin I, ERB, hsReq*-1 and hsReq*-2.

(52) A methodology of treating or preventing a disease or disorder involving aberrant levels of a complex of a CDK2 protein and a CDK2 protein-IP, wherein the CDK2 protein-IP is selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, within a subject, comprised of administering to a subject in which such treatment or prevention is desired, a

therapeutically-effective amount of a molecule or molecules which are capable of modulating the function of said complex.

(53) The methodology of claim 52, wherein said disease or disorder involves decreased levels of said complex and said molecule or molecules are capable of promoting the function of the complex of a CDK2 protein and a CDK2 protein-IP, and wherein said molecule or molecules are selected from the group consisting of: (*i*) a complex of a CDK2 protein and CDK2 protein-IP; (*ii*) a derivative or analog of a complex of a CDK2 protein and a CDK2 protein-IP which is more stable or more active than the wild-type complex; (*iii*) nucleic acids encoding a CDK2 protein and a CDK2 protein-IP and (*iv*) nucleic acids encoding a derivative or analog of a CDK2 protein and a CDK2 protein-IP which is capable of forming a complex which is more stable or more active than the wild-type complex.

(54) The methodology of claim 52, wherein said disease or disorder involves increased levels of said complex and said molecule or molecules are capable of inhibiting the function of the complex of a CDK2 protein and a CDK2 protein-IP, and wherein said molecule or molecules are selected from the group consisting of: (*i*) an antibody against said complex , or a fragment or derivative thereof, containing the binding region thereof; (*ii*) a CDK2 protein and a CDK2 protein-IP antisense nucleic acids and (*iii*) nucleic acids comprising, at least, a portion of a CDK2 protein and a CDK2 protein-IP gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of, at least, a portion of the CDK2 protein and CDK2 protein-IP genes, and wherein the CDK2 protein and the CDK2 protein-IP gene portions flank the heterologous sequences so as to promote homologous recombination with the genomic CDK2 protein and CDK2 protein-IP genes.

(55) A methodology of treating or preventing a disease or disorder involving an aberrant level of a CDK2 protein-IP selected from the group consisting of: hsReq*-1 and hsReq*-2, within a subject, which is comprised of administering to the subject in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule or molecules which modulates the function of said CDK2 protein-IP.

(56) The methodology of claim 55, wherein said disease or disorder involves a decreased level of a CDK2 protein-IP and said molecule or molecules promote the function of the CDK2 protein-IP; and wherein said molecule or molecules are selected from the group consisting of: (*i*) the CDK2 protein-IP protein; (*ii*) a derivative or analog of the CDK2 protein-IP which is capable of binding a CDK2 protein; (*iii*) a nucleic acid encoding the CDK2 protein-IP protein and (*iv*) a nucleic acid encoding a derivative or analog of the CDK2 protein-IP which is capable of binding a CDK2 protein.

(57) The methodology of claim 55, wherein said disease or disorder involves an increased level of the CDK2 protein-IP and said molecule or molecules inhibits the function of the CDK2 protein-IP; and wherein said molecule or molecules are selected from the group consisting of: (*i*) an anti-CDK2 protein-IP antibody, or a fragment or derivative thereof containing the binding region thereof; (*ii*) a CDK2 protein-IP antisense nucleic acid and (*iii*) a nucleic acid comprising, at least, a portion of the CDK2 protein-IP gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of, at least, a portion of the CDK2 protein-IP gene; wherein the CDK2 protein-IP gene portion flanks the heterologous sequence so as to promote homologous recombination with the genomic CDK2 protein-IP gene.

(58) A methodology for screening a purified complex of a CDK2 protein and a CDK2 protein-IP selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, or a derivative of said complex, or a modulator of the activity of said complex, for anti-neoplastic activity; wherein said methodology is comprised of measuring the survival or proliferation of cells from a cell line which is derived from, or displays characteristics associated with, malignant disorder; wherein said cells have been contacted with the complex, derivative, or modulator, compaired with the level of said indicator measured in cells not so contacted; and wherein a lower level in said contacted cells indicates that the complex, derivative or modulator possesses anti-neoplastic activity.

(59) A methodology for screening a purified complex of a CDK2 protein and a CDK2 protein-IP selected from the group consisting of: cyclin I. ERB. hsReq*-1 and hsReq*-2. or a derivative of said complex. or a modulator of the activity of said complex. for anti-neoplastic activity; wherein said methodology is comprised of administering the complex. derivative or modulator to a test animal which has a tumor, or which does not have a tumor but is subsequently challenged with tumor cells or tumorigenic agents. and measuring tumor growth or regression in said test animal; wherein decreased tumor growth or increased tumor regression or prevention of tumor growth within said test animals which were administered the complex. derivative, or modulator. in comparison to said test animals not so administered. indicates that the complex. derivative or modulator possesses anti-neoplastic activity.

(60) A method for screening a purified complex of CDK2 and a CDK2-IP selected from the group consisting of cyclin I. ERH. hsReq*-1, and hsReq*-2. or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing atherosclerosis or atherosclerosis-associated disease comprising contacting cultured cells that exhibit an indicator of an atherosclerotic reaction *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing atherosclerosis or atherosclerosis-associated diseases.

(61) A method for screening a purified complex of CDK2 and a CDK2-IP selected from the group consisting of cyclin I, ERH, hsReq*-1, and hsReq*-2, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing atherosclerosis or atherosclerosis-associated disease comprising administering said complex, derivative or modulator to a test animal, which test animal exhibits an atherosclerotic reaction, or which test animal does not exhibit an atherosclerotic reaction and is subsequently challenged with an agent that elicits an atherosclerotic reaction; and measuring the change in the atherosclerotic reaction after the administration of said complex. derivative or modulator. wherein a reduction in said atherosclerotic reaction or prevention of said atherosclerotic reaction indicates that the complex,

derivative or modulator has activity in treating or preventing atherosclerosis or an atherosclerosis-associated disease.

(62) A methodology of screening for a molecule or molecules which modulate, directly or indirectly, the formation of a complex of a CDK2 protein and a CDK2 protein-IP, in which said CDK2 protein-IP is selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2; wherein said methodology is comprised of measuring the levels of said complex formed from a CDK2 protein and a CDK2 protein-IP in the presence of said molecule or molecules under conditions which are conducive to formation of said complex, and comparing the levels of said complex with those levels of said complex which are formed in the absence of said molecule or molecules; wherein a lower or higher level of said complex in the presence of said molecule or molecules indicates that the molecule or molecules possess the ability to modulate the formation of said complex.

(63) A recombinant, non-human animal, or ancestor thereof, wherein both an endogenous CDK2 protein gene and an endogenous CDK2 protein-IP gene selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2 have been deleted or inactivated by homologous recombination or insertional mutagenesis.

(64) A recombinant, non-human animal, or ancestor thereof, containing both a CDK2 protein gene and a CDK2 protein-IP gene selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, wherein the CDK2 protein gene is under the control of a promoter which is not the promoter of the native CDK2 protein gene and the CDK2 protein-IP gene is under the control of a promoter which is not the promoter of the native CDK2 protein-IP gene.

(65) A recombinant, non-human animal, or ancestor thereof, containing a transgene comprising a nucleic acid sequence encoding the chimeric protein of claim 7.

(66) A recombinant, non-human animal, or ancestor thereof, containing a transgene comprising the nucleotide sequence of SEQ ID NO.:10; and SEQ ID NO:10.

(67) A methodology of modulating the activity or levels of a CDK2 protein: wherein said methodology is comprised of contacting a cell with, or administering to, an animal expressing a CDK2 protein gene, a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, or a nucleic acid encoding said protein, or an antibody which immunospecifically-binds said protein, or a fragment or derivative of said antibody containing the binding domain thereof.

(68) A methodology of modulating the activity or levels of a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2; wherein said methodology is comprised of contacting a cell with, or administering to, an animal expressing a gene encoding said protein, a CDK2 protein, a nucleic acid encoding a CDK2 protein, or an antibody which immunospecifically-binds a CDK2 protein, or a fragment or derivative of said antibody containing the binding domain thereof.

(69) A methodology of modulating the activity or levels of a complex of a CDK2 protein and a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2; wherein said methodology is comprised of contacting a cell with, or administering to an animal expressing and forming said complex, a molecule which possesses the ability to modulate the formation of said complex.

(70) A methodology for identifying a molecule which possesses the ability to modulate the activity of a CDK2 protein or a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, or a complex of a CDK2 protein and said protein; wherein said methodology is comprised of contacting one or more candidate molecules with a CDK2 protein in the presence of said protein, and measuring the amount of complex which forms between the CDK2 protein and said protein; and wherein an increase or decrease in the amount of complex which forms, relative to the amount of complex which forms in the absence of the candidate molecule or molecules, indicates that the molecule or molecules possess the ability to modulate the activity of a CDK2 protein, said protein or said complex of a CDK2 protein and said protein.

(71) The methodology of claim 70, wherein said contacting is carried out by administering the candidate molecule or molecules to the recombinant, non-human animal, or ancestor thereof, of claim 65.

(72) The methodology of claim 70, wherein said contacting is carried out *in vitro*; and the CDK2 protein, said protein, and said candidate molecule or molecules are purified.

(73) A methodology for screening a derivative or analog of a CDK2 protein for biological activity; wherein said methodology is comprised of contacting said derivative or analog of the CDK2 protein with a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, and detecting the formation of a complex between said derivative or analog of the CDK2 protein and said protein; and wherein detecting formation of said complex, indicates that said derivative or analog of the CDK2 protein possesses biological activity.

(74) A methodology for screening a derivative or analog of a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, for biological activity; wherein said methodology is comprised of contacting said derivative or analog of said protein with the CDK2 protein, and detecting the formation of a complex between said derivative or analog of said protein and the CDK2 protein; and wherein detecting the formation of said complex, indicates that said derivative or analog of said protein possesses biological activity.

(75) A methodology of monitoring the efficacy of a treatment of a disease or disorder characterized by an aberrant level of a complex of a CDK2 protein and a CDK2 protein-IP, in a subject which is administered said treatment for said disease or disorder; wherein said methodology is comprised of measuring the level of said complex, the RNAs encoding the CDK2 protein and the CDK2 protein-IP or the functional activity of said complex within a sample derived from said subject, wherein said sample is taken from said subject after the administration of said treatment, and compared to (*i*) said level within a sample taken from said subject prior to the administration of the treatment or (*ii*) a standard level associated with the pre-treatment stage of the disease or disorder; and wherein the change, or lack of change, in the level of said complex, said RNAs encoding the CDK2 protein and the CDK2 protein-IP or functional

activity of said complex within said sample taken after the administration of said treatment, relative to the level of said complex, said RNAs encoding the CDK2 protein and the CDK2 protein-IP or functional activity of said complex within said sample taken before the administration of said treatment, or to said standard level, indicates whether said administration is effective in the treatment of said disease or disorder.

(76) A methodology of treating or preventing cancer, or a hyperproliferative disorder, within a subject; wherein said methodology is comprised of administering to a subject, in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule or molecules which possess the ability to modulate the function of a complex of the CDK2 protein and a CDK2 protein-IP selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, or a combination of one or more of the aforementioned CDK2 protein-IPs.

(77) A methodology of treating or preventing atherosclerosis within a subject; wherein said methodology is comprised of administering to a subject, in which such treatment or prevention is desired, a therapeutically-effective amount of a molecule or molecules which possesses the ability to modulate the function of a complex of the CDK2 protein and a CDK2 protein-IP selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2, or a combination of one or more of the aforementioned CDK2 protein-IPs.

(78) A purified fragment of a protein selected from the group consisting of: cyclin I, ERH, hsReq*-1 and hsReq*-2; wherein said fragment possesses the ability to bind the CDK2 protein.

```
ATG GAG AAC TTC CAA AAG GTG GAA AAG ATC GGA GAG GGC ACG TAC GGA 48
Met Glu Asn Phe Gln Lys Val Glu Lys Ile Gly Glu Gly Thr Tyr Gly
 1               5                  10                  15

GTT GTG TAC AAA GCC AGA AAC AAG TTG ACG GGA GAG GTG GTG GCG CTT 96
Val Val Tyr Lys Ala Arg Asn Lys Leu Thr Gly Glu Val Val Ala Leu
            20                  25                  30

AAG AAA ATC CGC CTG GAC ACT GAG ACT GAG GGT GTG CCC AGT ACT GCC 144
Lys Lys Ile Arg Leu Asp Thr Glu Thr Glu Gly Val Pro Ser Thr Ala
        35                  40                  45

ATC CGA GAG ATC TCT CTG CTT AAG GAG CTT AAC CAT CCT AAT ATT GTC 192
Ile Arg Glu Ile Ser Leu Leu Lys Glu Leu Asn His Pro Asn Ile Val
    50                  55                  60

AAG CTG CTG GAT GTC ATT CAC ACA GAA AAT AAA CTC TAC CTG GTT TTT 240
Lys Leu Leu Asp Val Ile His Thr Glu Asn Lys Leu Tyr Leu Val Phe
65                  70                  75                  80

GAA TTT CTG CAC CAA GAT CTC AAG AAA TTC ATG GAT GCC TCT GCT CTC 288
Glu Phe Leu His Gln Asp Leu Lys Lys Phe Met Asp Ala Ser Ala Leu
                85                  90                  95

ACT GGC ATT CCT CTT CCC CTC ATC AAG AGC TAT CTG TTC CAG CTG CTC 336
Thr Gly Ile Pro Leu Pro Leu Ile Lys Ser Tyr Leu Phe Gln Leu Leu
            100                 105                 110

CAG GGC CTA GCT TTC TGC CAT TCT CAT CGG GTC CTC CAC CGA GAC CTT 384
Gln Gly Leu Ala Phe Cys His Ser His Arg Val Leu His Arg Asp Leu
        115                 120                 125

AAA CCT CAG AAT CTG CTT ATT AAC ACA GAG GGG GCC ATC AAG CTA GCA 432
Lys Pro Gln Asn Leu Leu Ile Asn Thr Glu Gly Ala Ile Lys Leu Ala
    130                 135                 140

GAC TTT GGA CTA GCC AGA GCT TTT GGA GTC CCT GTT CGT ACT TAC ACC 480
Asp Phe Gly Leu Ala Arg Ala Phe Gly Val Pro Val Arg Thr Tyr Thr
145                 150                 155                 160

CAT GAG GTG GTG ACC CTG TGG TAC CGA GCT CCT GAA ATC CTC CTG GGC 528
His Glu Val Val Thr Leu Trp Tyr Arg Ala Pro Glu Ile Leu Leu Gly
                165                 170                 175

TCG AAA TAT TAT TCC ACA GCT GTG GAC ATC TGG AGC CTG GGC TGC ATC 576
Ser Lys Tyr Tyr Ser Thr Ala Val Asp Ile Trp Ser Leu Gly Cys Ile
            180                 185                 190

TTT GCT GAG ATG GTG ACT CGC CGG GCC CTG TTC CCT GGA GAT TCT GAG 624
Phe Ala Glu Met Val Thr Arg Arg Ala Leu Phe Pro Gly Asp Ser Glu
        195                 200                 205
```

Fig. 1

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ATT GAC CAG CTC TTC CGG ATC TTT CGG ACT CTG GGG ACC CCA GAT GAG 672
Ile Asp Gln Leu Phe Arg Ile Phe Arg Thr Leu Gly Thr Pro Asp Glu
    210                 215                 220

GTG GTG TGG CCA GGA GTT ACT TCT ATG CCT GAT TAC AAG CCA AGT TTC 720
Val Val Trp Pro Gly Val Thr Ser Met Pro Asp Tyr Lys Pro Ser Phe
225                 230                 235                 240

CCC AAG TGG GCC CGG CAA GAT TTT AGT AAA GTT GTA CCT CCC CTG GAT 768
Pro Lys Trp Ala Arg Gln Asp Phe Ser Lys Val Val Pro Pro Leu Asp
                245                 250                 255

GAA GAT GGA CGG AGC TTG TTA TCG CAA ATG CTG CAC TAC GAC CCT AAC 816
Glu Asp Gly Arg Ser Leu Leu Ser Gln Met Leu His Tyr Asp Pro Asn
            260                 265                 270

AAG CGG ATT TCG GCC AAG GCA GCC CTG GCT CAC CCT TTC TTC CAG GAT 864
Lys Arg Ile Ser Ala Lys Ala Ala Leu Ala His Pro Phe Phe Gln Asp
        275                 280                 285

GTG ACC AAG CCA GTA CCC CAT CTT CGA CTC TGATAGCCTT CTTGAAGCCC CCG 917
Val Thr Lys Pro Val Pro His Leu Arg Leu
    290                 295         298

ACCCTAATCG GCTCACCCTC TCCTCCAGTG TGGGCTTGAC CAGCTTGGCC TTGGGCTATT  977

TGGACTCAGG TGGGCCCTCT GAACTTGCCT TAAACACTCA CCTTCTAGTC TTAACCAGCC 1037

AACTCTGGGA ATACAGGGGT GAAAGGGGGG AACCAGTGAA AATGAAAGGA AGTTTCAGTA 1097

TTAGATGCAC TTAAGTTAGC CTCCACCACC CTTTCCCCCT TCTCTTAGTT ATTGCTGAAG 1157

AGGGTTGGTA TAAAAATAAT TTTAAAAAAG CCTTCCTACA CGTTAGATTT GCCGTACCAA 1217

TCTCTGAATG CCCCATAATT ATTATTTCCA GTGTTTGGGA TGACCAGGAT CCCAAGCCTC 1277

CTGCTGCCAC AATGTTTATA AAGGCCAAAT GATAGCGGGG GCTAAGTTGG TGCTTTTGAG 1337

AATTAAGTAA AACAAAACCA CTGGGAGGAG TCTATTTTAA AGAATTCGGT TAAAAAATAG 1397

ATCCAATCAG TTTATACCCT AGTTAGTGTT TTCCTCACCT AATAGGCTGG GAGACTGAAG 1457

ACTCAGCCCG GGTGGGGGT                                              1476
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Fig. 1 (continued)

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ATG AAG TTT CCA GGG CCT TTG GAA AAC CAG AGA TTG TCT TTC CTG TTG  48
Met Lys Phe Pro Gly Pro Leu Glu Asn Gln Arg Leu Ser Phe Leu Leu
 1               5                  10                  15

GAA AAG GCA ATC ACT AGG GAA GCA CAG ATG TGG AAA GTG AAT GTG CGG  96
Glu Lys Ala Ile Thr Arg Glu Ala Gln Met Trp Lys Val Asn Val Arg
            20                  25                  30

AAA ATG CCT TCA AAT CAG AAT GTT TCT CCA TCC CAG AGA GAT GAA GTA 144
Lys Met Pro Ser Asn Gln Asn Val Ser Pro Ser Gln Arg Asp Glu Val
        35                  40                  45

ATT CAA TGG CTG GCC AAA CTC AAG TAC CAA TTC AAC CTT TAC CCA GAA 192
Ile Gln Trp Leu Ala Lys Leu Lys Tyr Gln Phe Asn Leu Tyr Pro Glu
    50                  55                  60

ACA TTT GCT CTG GCT AGC AGT CTT TTG GAT AGG TTT TTA GCT ACC GTA 240
Thr Phe Ala Leu Ala Ser Ser Leu Leu Asp Arg Phe Leu Ala Thr Val
65                  70                  75                  80

AAG GCT CAT CCA AAA TAC TTG AGT TGT ATT GCA ATC AGC TGT TTT TTC 288
Lys Ala His Pro Lys Tyr Leu Ser Cys Ile Ala Ile Ser Cys Phe Phe
                85                  90                  95

CTA GCT GCC AAG ACT GTT GAG GAA GAT GAG AGA ATT CCA GTA CTA AAG 336
Leu Ala Ala Lys Thr Val Glu Glu Asp Glu Arg Ile Pro Val Leu Lys
            100                 105                 110

GTA TTG GCA AGA GAC AGT TTC TGT GGA TGT TCC TCA TCT GAA ATT TTG 384
Val Leu Ala Arg Asp Ser Phe Cys Gly Cys Ser Ser Ser Glu Ile Leu
        115                 120                 125

AGA ATG GAG AGA ATT ATT CTG GAT AAG TTG AAT TGG GAT CTT CAC ACA 432
Arg Met Glu Arg Ile Ile Leu Asp Lys Leu Asn Trp Asp Leu His Thr
    130                 135                 140

GCC ACA CCA TTG GAT TTT CTT CAT ATT TTC CAT GCC ATT GCA GTG TCA 480
Ala Thr Pro Leu Asp Phe Leu His Ile Phe His Ala Ile Ala Val Ser
145                 150                 155                 160

ACT AGG CCT CAG TTA CTT TTC AGT TTG CCC AAA TTG AGC CCA TCT CAA 528
Thr Arg Pro Gln Leu Leu Phe Ser Leu Pro Lys Leu Ser Pro Ser Gln
                165                 170                 175

CAT TTG GCA GTC CTT ACC AAG CAA CTA CTT CAC TGT ATG GCC TGC AAC 576
His Leu Ala Val Leu Thr Lys Gln Leu Leu His Cys Met Ala Cys Asn
            180                 185                 190
```

Fig. 2

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CAA CTT CTG CAA TTC AGA GGA TCC ATG CTT GCT CTG GCC ATG GTT AGT  624
Gln Leu Leu Gln Phe Arg Gly Ser Met Leu Ala Leu Ala Met Val Ser
        195                 200                 205

CTG GAA ATG GAG AAA CTC ATT CCT GAT TGG CTT TCT CTT ACA ATT GAA  672
Leu Glu Met Glu Lys Leu Ile Pro Asp Trp Leu Ser Leu Thr Ile Glu
    210                 215                 220

CTG CTT CAG AAA GCA CAG ATG GAT AGC TCC CAG TTG ATC CAT TGT CGG  720
Leu Leu Gln Lys Ala Gln Met Asp Ser Ser Gln Leu Ile His Cys Arg
225                 230                 235                 240

GAG CTT GTG GCA CAT CAC CTT TCT ACT CTG CAG TCT TCC CTG CCT CTG  768
Glu Leu Val Ala His His Leu Ser Thr Leu Gln Ser Ser Leu Pro Leu
                245                 250                 255

AAT TCC GTT TAT GTC TAC CGT CCC CTC AAG CAC ACC CTG GTG ACC TGT  816
Asn Ser Val Tyr Val Tyr Arg Pro Leu Lys His Thr Leu Val Thr Cys
            260                 265                 270

GAC AAA GGA GTG TTC AGA TTA CAT CCC TCC TCT GTC CCA GGC CCA GAC  864
Asp Lys Gly Val Phe Arg Leu His Pro Ser Ser Val Pro Gly Pro Asp
        275                 280                 285

TTC TCC AAG GAC AAC AGC AAG CCA GAA GTG CCA GTC AGA GGT ACA GCA  912
Phe Ser Lys Asp Asn Ser Lys Pro Glu Val Pro Val Arg Gly Thr Ala
    290                 295                 300

GCC TTT TAC CAT CAT CTC CCA GCT GCC AGT GGG TGC AAG CAG ACC TCT  960
Ala Phe Tyr His His Leu Pro Ala Ala Ser Gly Cys Lys Gln Thr Ser
305                 310                 315                 320

ACT AAA CGC AAA GTA GAG GAA ATG GAA GTG GAT GAC TTC TAT GAT GGA 1008
Thr Lys Arg Lys Val Glu Glu Met Glu Val Asp Asp Phe Tyr Asp Gly
                325                 330                 335

ATC AAA CGG CTC TAT AAT GAA GAT AAT GTC TCA GAA AAT GTG GGT TCT 1056
Ile Lys Arg Leu Tyr Asn Glu Asp Asn Val Ser Glu Asn Val Gly Ser
            340                 345                 350

GTG TGT GGC ACT GAT TTA TCA AGA CAA GAG GGA CAT GCT TCC CCT TGT 1104
Val Cys Gly Thr Asp Leu Ser Arg Gln Glu Gly His Ala Ser Pro Cys
        355                 360                 365

CCA CCT TTG CAG CCT GTT TCT GTC ATG TAGTTTCAAC AAGTGCTACC TTTGAGT 1158
Pro Pro Leu Gln Pro Val Ser Val Met
    370                 375     377

GTAAACTAAG GTAGACTACT TTGGGAATGA GAACATCCAA AATCAGGAAA GGCTGTAGAA 1218

GGAAATATAC CTTAACAGGC TGATTTGGAG TGACCCAGAA AA                    1260
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Fig. 2 (continued)

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GGCACGAGGT TGTAGTTAAG CTCGTGTAAC GGCGGCGGTG TCGGTAGCTG CTGTAGCGAA 60

GAGAGTTTGG CGCG ATG TCT CAC ACC ATT TTG CTG GTA CAG CCT ACC AAG  110
                Met Ser His Thr Ile Leu Leu Val Gln Pro Thr Lys
                 1               5                  10
                                                    ⬇
AGG CCA GAA GGC AGA ACT TAT GCT GAC TAC GAA TCT GTG AAT GAA TGC  158
Arg Pro Glu Gly Arg Thr Tyr Ala Asp Tyr Glu Ser Val Asn Glu Cys
        15                  20                  25

ATG GAA GGT GTT TGT AAA ATG TAT GAA GAA CAT CTG AAA AGA ATG AAT  206
Met Glu Gly Val Cys Lys Met Tyr Glu Glu His Leu Lys Arg Met Asn
    30                  35                  40

CCC AAC AGT CCC TCT ATC ACA TAT GAC ATC AGT CAG TTG TTT GAT TTC  254
Pro Asn Ser Pro Ser Ile Thr Tyr Asp Ile Ser Gln Leu Phe Asp Phe
45                  50                  55                  60

ATC GAT GAT CTG GCA GAC CTC AGC TGC CTG GTT TAC CGA GCT GAT ACC  302
Ile Asp Asp Leu Ala Asp Leu Ser Cys Leu Val Tyr Arg Ala Asp Thr
                65                  70                  75

CAG ACA TAC CAG CCT TAT AAC AAA GAC TGG ATT AAA GAG AAG ATC TAC  350
Gln Thr Tyr Gln Pro Tyr Asn Lys Asp Trp Ile Lys Glu Lys Ile Tyr
            80                  85                  90

GTG CTC CTT CGT CGG CAG GCC CAA CAG GCT GGG AAA TAATTGTGTT GGAAGC 402
Val Leu Leu Arg Arg Gln Ala Gln Gln Ala Gly Lys
        95                  100                 104

ACTGGGGGGG TTGGGGTGGG CTTGGAACAC AGGTGTGTAC AGCGTGCTGT AGTGGAAGTT 462
TTGTATCATA GTAATCCTGT TTCCACTTTG TTATACTCTA GCCAAGATTG ACTGTATTAG 522
ATGAAATGTG AGGATCTTGT TCAATCGGAA ACCCCCGTTA CCTCCTCTTT TTCTTTCTCT 582
TTCTTTTTTT TTTTTTACTT AAACATTTTT ATGATGATTT AGATGGAAGT TGTTCTTCGT 642
CACTTAATGT TGGTTCCAGT CCTTCAACTG TTCATATCTA CTTTATAACA TTCACATACT 702
AACCCTTCTG GGGTTCAAGA TGGGGGGTGG CAAATGCAGT TTAGCCATGT CCTCAAGATA 762
AAGTCTTGGT AAAAATAAAT AAATGTCCTT TAGTT                           797
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Fig. 3

1 GGAAGATGGC GGCTGTGGTG GAGAATGTAG TGAAGCTCCT TGGGGAGCAG TACTACAAAG
61 ATGCCATGGA GCAGTGCCAC AATTACAATG CTCGCCTCTG TGCTGAGCGC AGCGTGCGCC
121 TGCCTTTCTT GGACTCACAG ACCGGAGTAG CCCAGAGCAA TTGTTACATC TGGATGGAAA
181 AGCGACACCG GGGTCCAGGA TTGGCCTCCG GACAGCTGTA CTCCTACCCT GCCCGGCGCT
241 GGCGGAAAAA GCGGCGAGCC CATCCCCCTG AGGATCCACG ACTTTCCTTC CCATCTATTA
301 AGCCAGACAC AGACCAGACC CTGAAGAAGG AGGGGCTGAT CTCTCAGGAT GGCAGTAGTT
361 TAGAGGCTCT GTTGCGCACT GACCCCCTGG AGAAGCGAGG TGCCCCGGAT CCCCGAGTTG
421 ATGATGACAG CCTGGGCGAG TTTCCTGTGA CCAACAGTCG AGCGCGAAAG CGGATCCTAG
481 AACCAGATGA CTTCCTGGAT GACCTCGATG ATGAAGACTA TGAAGAAGAT ACTCCCAAGC
541 GTCGGGGAAA GGGGAAATCC AAGGGTAAGG GTGTGGGCAG TGCCCGTAAG AAGCTGGATG
601 CTTCCATCCT GGAGGACCGG GATAAGCCCT ATGCCTGTGA CATTTGTGGA AAACGTTACA
661 AGAACCGACC AGGCCTCAGT TACCACTATG CCCACTCCCA CTTGGCTGAG GAGGAGGGCG
721 AGGACAAGGA AGACTCTCAA CCACCCACTC CTGTTTCCCA GAGGTCTGAG GAGCAGAAAT
781 CCAAAAAGGG TCCTGATGGA TTGGCCTTGC CCAACAACTA CTGTGACTTC TGCCTGGGGG
841 ACTCAAAGAT TAACAAGAAG ACGGGACAAC CCGAGGAGCT GGTGTCTTGT TCTGACTGTG
901 GCCGCTCAGG GCATCCATCT TGCCTCCAAT TTACCCCCGT GATGATGGCG GCAGTGAAGA
961 CATACCGCTG GCAGTGCATC GAGTGCAAAT GTTGCAATAT CTGCGGCACC TCCGAGAATG
1021 ACGACCAGTT GCTCTTCTGT GATGACTGCG ATCGTGGCTA CCACATGTAC TGTCTCACCC
1081 CGTCCATGTC TGAGCCCCCT GAAGGAAGTT GGAGCTGCCA CCTGTGTCTG GACCTGTTGA
1141 AAGAGAAAGC TTCCATCTAC CAGAACCAGA ACTCCTCTTG ATGTGGCCAC CCACCTGCTC
1201 CCCGACATAT CTAAGGCTGT TTCTCTCCTC CACTTCATAT TTCATACCCA TCTTTCCCTT

Fig. 4

1261 CTTCCTCCTC TCCTTCACAA ATCCAGAGAA CCTTGGGGTG GTTGTGCCAG CCTGCCTTTG

1321 GCAGCTGCAA GCTGAGGTGG CAGCTCTGAC CACCTCTGGC CCCAGGCCTC AGGGAGAAAG

1381 GAGCAACACA CTGCCCCTAG GCGTGCGTGT GGCCCAGTTT CTCTCTGCTC TCCATTAAGT

1441 GCATTCACTC TGCTTGCCTT GGGCCCAGCC CCTGGTGATC ACAGGGTTCA AACAGTGTCC

1501 TCCTAGAAAG AGTGGGAGAG CAGCTCACTT CTCTGTGTTC TGCCTCCCCT CTGGTCTCCA

1561 GAGTTTTCCT GTCCTCTAGA GGCAAGCCAG GCCAGGGAGC TGGGAGCGAG CAAGCTGAGG

1621 CCACGTCCAC AAGGAGCTTT TCATGCCCCT GTGCCGCATA GCCTCACCTC TTTCCTCCAG

1681 AGTGGCTCTC TGCGGCCCTG TGTTCCTGCT ACAGAGTGTT CTTTTCTGGA GTCAGGATGT

1741 TCTCGGTCAC CCTCCTGGTT CTGCCCTGTC CCATTCCACC CCACCCCAGG GGGAACAGTA

1801 GCTTCACCTT GTTATTCCCA TTGCTCTCCT GGCTCACTCT TACGGTCGGT CTCCAGTGAC

1861 TGAAGCATTC CCCACCCTTG GAATTTCTCA TCTTCTGCCT CCCTTCCTAC TCCTTTTGGT

1921 TTTGTGGGGA GAGGGGAAGG ATCAGGGGGC AAGGCCAGCA GCTCGGGGGC CACAAGGAGA

1981 TGGATAATGT GCCTGTTTTT TAACACAACA AAAAAGCCTA CCTCCAAAAT CCCCTTTTTG

2041 TTCTTCCTGG ACCTGGGCAT TCAGCCTCCT GCTCTTAACT GAATTGGGAG CCTCTGCCAC

2101 CTGCCCCGTG TATCCTGGCT CTCAGCTCAT GGGGAAGCCA CATAGACATC CCTTTCTTCC

2161 CTTGCACGCT CGCTAGCAGC TGGTAGGTCT TCACACCCTG ATTCCTCAAG TTTTCTGCTT

2221 AGTGGCACTG ACATTAAGTA GTGGGGGGAC AGTCCATGCC AGGACACCCT GGAGTAGCCT

2281 TCCCCCTTGG CCGTGGGCAG GCCCTAACTC ACTGTCGCTT TGGAGTTGAG GTGTCTTTTT

2341 TTTTCTTTC TTTAGTTCCT GTATTCTAAA CATTAGTAAA AATAAATGTT TTTACACAG 2400

Fig. 4 (continued)

```
  1 ATG GCG GCT GTG GTG GAG AAT GTA GTG AAG CTC CTT GGG GAG CAG
    Met Ala Ala Val Val Glu Gln Val Val Lys Leu Leu Gly Glu Gln
    1               5                  10                  15

 46 TAC TAC AAA GAT GCC ATG GAG CAG TGC CAC AAT TAC AAT GCT CGC
    Tyr Tyr Lys Asp Ala Met Glu Gln Cys His Asn Tyr Asn Ala Arg
                    20                  25                  30

 91 CTC TGT GCT GAG CGC AGC GTG CGC CTG CCT TTC TTG GAC TCA CAG
    Leu Cys Ala Glu Arg Ser Val Arg Leu Pro Phe Leu Asp Ser Gln
                    35                  40                  45

136 ACC GGA GTA GCC CAG AGC AAT TGT TAC ATC TGG ATG GAA AAG CGA
    Thr Gly Val Ala Gln Ser Asn Cys Tyr Ile Trp Met Glu Lys Arg
                    50                  55                  60

181 CAC CGG GGT CCA GGA TTG GCC TCC GGA CAG CTG TAC TCC TAC CCT
    His Arg Gly Pro Gly Leu Ala Ser Gly Gln Ley Tyr Ser Tyr Pro
                    65                  70                  75

226 GCC CGG CGC TGG CGG AAA AAG CGG CGA GCC CAT CCC CCT GAG GAT
    Ala Arg Arg Trp Arg Lys Lys Arg Arg Ala His Pro Pro Glu Asp
                    80                  85                  90

271 CCA CGA CTT TCC TTC CCA TCT ATT AAG CCA GAC ACA GAC CAG ACC
    Pro Arg Leu Ser Phe Pro Ser Ile Lys Pro Asp Thr Asp Gln Thr
                    95                  100                 105

316 CTG AAG AAG GAG GGG CTG ATC TCT CAG GAT GGC AGT AGT TTA GAG
    Leu Lys Lys Glu Gly Leu Ile Ser Gln Asp Gly Ser Ser Leu Glu
                    110                 115                 120
```

Fig. 5

```
361 GCT CTG TTG CGC ACT GAC CCC CTG GAG AAG CGA GGT GCC CCG GAT
    Ala Leu Leu Arg Thr Asp Pro Leu Glu Lys Arg Gly Ala Pro Asp
                    125                 130                 135

406 CCC CGA GTT GAT GAT GAC AGC CTG GGC GAG TTT CCT GTG ACC AAC
    Pro Arg Val Asp Asp Asp Ser Leu Gly Glu Phe Pro Val Thr Asn
                    140                 145                 150

451 AGT CGA GCG CGA AAG CGG ATC CTA GAA CCA GAT GAC TTC CTG GAT
    Ser Arg Ala Arg Lys Arg Ile Leu Glu Pro Asp Asp Phe Leu Asp
                    155                 160                 165

496 GAC CTC GAT GAT GAA GAC TAT GAA GAA GAT ACT CCC AAG CGT CGG
    Asp Leu Asp Asp Glu Asp Tyr Glu Glu Asp Thr Pro Lys Arg Arg
                    170                 175                 180
                                    ⬇Ⓐ
541 GGA AAG GGG AAA TCC AAG GAG GCA AGC CAG GCC AGG GAG CTG GGA
    Gly Lys Gly Lys Ser Lys Glu Ala Ser Gln Ala Arg Glu Leu Gly
                    185                 190                 195

586 GCG AGC AAG CTG AGG CCA CGT CCA CAA GGA GCT TTT CAT GCC CCT
    Ala Ser Lys Leu Arg Pro Arg Pro Gln Gly Ala Phe His Ala Pro
                    200                 205                 210

631 GTG CCG CAT AGC CTC ACC TCT TTC CTC CAG AGT GGC TCT CTG CGG
    Val Pro His Ser Leu Thr Ser Phe Leu Gln Ser Gly Ser Leu Arg
                    215                 220                 225
```

Fig. 5 (continued)

```
676 CCC TGT GTT CCT GCT ACA GAG TGT TCT TTT CTG GAG TCA GGA TGT
    Pro Cys Val Pro Ala Thr Glu Cys Ser Phe Leu Glu Ser Gly Cys
                    230                 235                 240

721 TCT CGG TCA CCC TCC TGG TTC TGC CCT GTC CCA TTC CAC CCC ACC
    Ser Arg Ser Pro Ser Trp Phe Cys Pro Val Pro Phe His Pro Thr
          ⬇Ⓑ        245                 250          ⬇Ⓒ     255
766 CCA GGG GGA ACA GTA GCT TCA CCT TGT TAT TCC CAT TGC TCT CCT
    Pro Gly Gly Thr Val Ala Ser Pro Cys Tyr Ser His Cys Ser Pro
                    260                 265                 270

811 GGC TCA CTC TTA CGG TCG GTC TCC AGT GAC TGA 843
    Gly Ser Leu Leu Arg Ser Val Ser Ser Asp *
                    275                 280
```

Fig. 5 (continued)

```
  1 ATG GCG GCT GTG GTG GAG AAT GTA GTG AAG CTC CTT GGG GAG CAG
    Met Ala Ala Val Val Glu Asn Val Val Lys Leu Leu Gly Glu Gln
    1               5                  10                  15

 46 TAC TAC AAA GAT GCC ATG GAG CAG TGC CAC AAT TAC AAT GCT CGC
    Tyr Tyr Lys Asp Ala Met Glu Gln Cys His Asn Tyr Asn Ala Arg
                    20                  25                  30

 91 CTC TGT GCT GAG CGC AGC GTG CGC CTG CCT TTC TTG GAC TCA CAG
    Leu Cys Ala Glu Arg Ser Val Arg Leu Pro Phe Leu Asp Ser Gln
                    35                  40                  45

136 ACC GGA GTA GCC CAG AGC AAT TGT TAC ATC TGG ATG GAA AAG CGA
    Thr Gly Val Ala Gln Ser ASN Cys Tyr Ile Trp Met Glu Lys Arg
                    50                  55                  60

181 CAC CGG GGT CCA GGA TTG GCC TCC GGA CAG CTG TAC TCC TAC CCT
    His Arg Gly Pro Gly Leu Ala Ser Gly Gln Leu Tyr Ser Tyr Pro
                    65                  70                  75

226 GCC CGG CGC TGG CGG AAA AAG CGG CGA GCC CAT CCC CCT GAG GAT
    Ala Arg Arg Trp Arg Lys Lys Arg Arg Ala His Pro Pro Glu Asp
                    80                  85                  90

271 CCA CGA CTT TCC TTC CCA TCT ATT AAG CCA GAC ACA GAC CAG ACC
    Pro Arg Leu Ser Phe Pro Ser Ile Lys Pro Asp Thr Asp Gln Thr
                    95                  100                 105

316 CTG AAG AAG GAG GGG CTG ATC TCT CAG GAT GGC AGT AGT TTA GAG
    Leu Lys Lys Glu Gly Leu Ile Ser Gln Asp Gly Ser Ser Leu Glu
                    110                 115                 120
```

Fig. 6

```
361 GCT CTG TTG CGC ACT GAC CCC CTG GAG AAG CGA GGT GCC CCG GAT
    Ala Leu Leu Arg Thr Asp Pro Leu Glu Lys Arg Gly Ala Pro Asp
                    125                 130                 135

406 CCC CGA GTT GAT GAT GAC AGC CTG GGC GAG TTT CCT GTG ACC AAC
    Pro Arg Val Asp Asp Asp Ser Leu Gly Glu Phe Pro Val Thr Asn
                    140                 145                 150

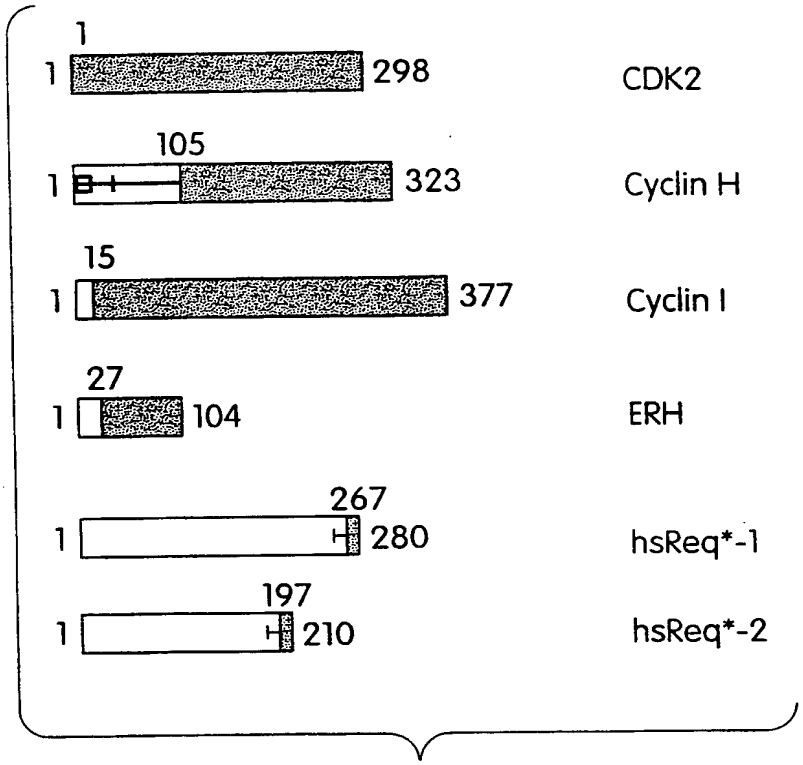
451 AGT CGA GCG CGA AAG CGG ATC CTA GAA CCA GAT GAC TTC CTG GAT
    Ser Arg Ala Arg Lys Arg Ile Glu Leu Pro Asp Asp Phe Leu Asp
                    155                 160                 165

496 GAC CTC GAT GAT GAA GAC TAT GAA GAA GAT ACT CCC AAG CGT CGG
    Asp Leu Asp Asp Glu Asp Tyr Glu Glu Asp Thr Pro Lys Arg Arg
                    170                 175                 180
                                    ⬇ (A)
541 GGA AAG GGG AAA TCC AAG GGG GGA ACA GTA GCT TCA CCT TGT TAT
    Gly Lys Gly Lys Ser Lys Gly Gly Thr Val Ala Ser Pro Cys Tyr
                    185                 190                 195
        ⬇ (B)
586 TCC CAT TGC TCT CCT GGC TCA CTC TTA CGG TCG GTC TCC AGT GAC
    Ser His Cys Ser Pro Gly Ser Leu Leu Arg Ser Val Ser Ser Asp
                    200                 205                 210

631 TGA 633

    *
```

Fig. 6 (continued)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1
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hsReq*-2

Fig. 7

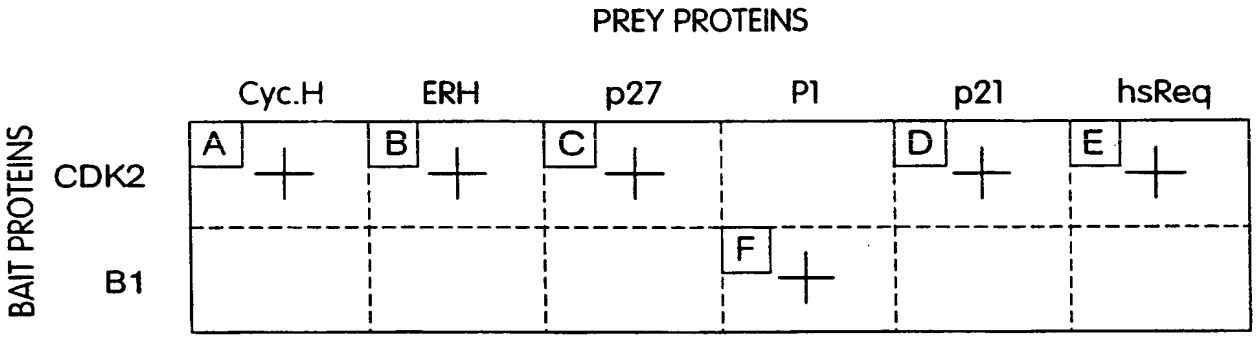

Fig. 8

```
ATG GAG AAC TTC CAA AAG GTG GAA AAG ATC GGA GAG GGC ACG TAC GGA 48
Met Glu Asn Phe Gln Lys Val Glu Lys Ile Gly Glu Gly Thr Tyr Gly
 1               5                  10                  15

GTT GTG TAC AAA GCC AGA AAC AAG TTG ACG GGA GAG GTG GTG GCG CTT 96
Val Val Tyr Lys Ala Arg Asn Lys Leu Thr Gly Glu Val Val Ala Leu
            20                  25                  30

AAG AAA ATC CGC CTG GAC ACT GAG ACT GAG GGT GTG CCC AGT ACT GCC 144
Lys Lys Ile Arg Leu Asp Thr Glu Thr Glu Gly Val Pro Ser Thr Ala
        35                  40                  45

ATC CGA GAG ATC TCT CTG CTT AAG GAG CTT AAC CAT CCT AAT ATT GTC 192
Ile Arg Glu Ile Ser Leu Leu Lys Glu Leu Asn His Pro Asn Ile Val
    50                  55                  60

AAG CTG CTG GAT GTC ATT CAC ACA GAA AAT AAA CTC TAC CTG GTT TTT 240
Lys Leu Leu Asp Val Ile His Thr Glu Asn Lys Leu Tyr Leu Val Phe
65                  70                  75                  80

GAA TTT CTG CAC CAA GAT CTC AAG AAA TTC ATG GAT GCC TCT GCT CTC 288
Glu Phe Leu His Gln Asp Leu Lys Lys Phe Met Asp Ala Ser Ala Leu
                85                  90                  95

ACT GGC ATT CCT CTT CCC CTC ATC AAG AGC TAT CTG TTC CAG CTG CTC 336
Thr Gly Ile Pro Leu Pro Leu Ile Lys Ser Tyr Leu Phe Gln Leu Leu
            100                 105                 110

CAG GGC CTA GCT TTC TGC CAT TCT CAT CGG GTC CTC CAC CGA GAC CTT 384
Gln Gly Leu Ala Phe Cys His Ser His Arg Val Leu His Arg Asp Leu
        115                 120                 125

AAA CCT CAG AAT CTG CTT ATT AAC ACA GAG GGG GCC ATC AAG CTA GCA 432
Lys Pro Gln Asn Leu Leu Ile Asn Thr Glu Gly Ala Ile Lys Leu Ala
    130                 135                 140

GAC TTT GGA CTA GCC AGA GCT TTT GGA GTC CCT GTT CGT ACT TAC ACC 480
Asp Phe Gly Leu Ala Arg Ala Phe Gly Val Pro Val Arg Thr Tyr Thr
145                 150                 155                 160

CAT GAG GTG GTG ACC CTG TGG TAC CGA GCT CCT GAA ATC CTC CTG GGC 528
His Glu Val Val Thr Leu Trp Tyr Arg Ala Pro Glu Ile Leu Leu Gly
                165                 170                 175

TCG AAA TAT TAT TCC ACA GCT GTG GAC ATC TGG AGC CTG GGC TGC ATC 576
Ser Lys Tyr Tyr Ser Thr Ala Val Asp Ile Trp Ser Leu Gly Cys Ile
            180                 185                 190

TTT GCT GAG ATG GTG ACT CGC CGG GCC CTG TTC CCT GGA GAT TCT GAG 624
Phe Ala Glu Met Val Thr Arg Arg Ala Leu Phe Pro Gly Asp Ser Glu
        195                 200                 205
```

Fig. 1

```
ATT GAC CAG CTC TTC CGG ATC TTT CGG ACT CTG GGG ACC CCA GAT GAG 672
Ile Asp Gln Leu Phe Arg Ile Phe Arg Thr Leu Gly Thr Pro Asp Glu
    210                 215                 220

GTG GTG TGG CCA GGA GTT ACT TCT ATG CCT GAT TAC AAG CCA AGT TTC 720
Val Val Trp Pro Gly Val Thr Ser Met Pro Asp Tyr Lys Pro Ser Phe
225                 230                 235                 240

CCC AAG TGG GCC CGG CAA GAT TTT AGT AAA GTT GTA CCT CCC CTG GAT 768
Pro Lys Trp Ala Arg Gln Asp Phe Ser Lys Val Val Pro Pro Leu Asp
                245                 250                 255

GAA GAT GGA CGG AGC TTG TTA TCG CAA ATG CTG CAC TAC GAC CCT AAC 816
Glu Asp Gly Arg Ser Leu Leu Ser Gln Met Leu His Tyr Asp Pro Asn
            260                 265                 270

AAG CGG ATT TCG GCC AAG GCA GCC CTG GCT CAC CCT TTC TTC CAG GAT 864
Lys Arg Ile Ser Ala Lys Ala Ala Leu Ala His Pro Phe Phe Gln Asp
        275                 280                 285

GTG ACC AAG CCA GTA CCC CAT CTT CGA CTC TGATAGCCTT CTTGAAGCCC CCG 917
Val Thr Lys Pro Val Pro His Leu Arg Leu
    290                 295         298

ACCCTAATCG GCTCACCCTC TCCTCCAGTG TGGGCTTGAC CAGCTTGGCC TTGGGCTATT  977

TGGACTCAGG TGGGCCCTCT GAACTTGCCT TAAACACTCA CCTTCTAGTC TTAACCAGCC 1037

AACTCTGGGA ATACAGGGGT GAAAGGGGGG AACCAGTGAA AATGAAAGGA AGTTTCAGTA 1097

TTAGATGCAC TTAAGTTAGC CTCCACCACC CTTTCCCCCT TCTCTTAGTT ATTGCTGAAG 1157

AGGGTTGGTA TAAAAATAAT TTTAAAAAAG CCTTCCTACA CGTTAGATTT GCCGTACCAA 1217

TCTCTGAATG CCCCATAATT ATTATTTCCA GTGTTTGGGA TGACCAGGAT CCCAAGCCTC 1277

CTGCTGCCAC AATGTTTATA AAGGCCAAAT GATAGCGGGG GCTAAGTTGG TGCTTTTGAG 1337

AATTAAGTAA AACAAAACCA CTGGGAGGAG TCTATTTTAA AGAATTCGGT TAAAAAATAG 1397

ATCCAATCAG TTTATACCCT AGTTAGTGTT TTCCTCACCT AATAGGCTGG GAGACTGAAG 1457

ACTCAGCCCG GGTGGGGGGT                                            1476
```

Fig. 1 (continued)

```
ATG AAG TTT CCA GGG CCT TTG GAA AAC CAG AGA TTG TCT TTC CTG TTG  48
Met Lys Phe Pro Gly Pro Leu Glu Asn Gln Arg Leu Ser Phe Leu Leu
 1               5                  10                  15

GAA AAG GCA ATC ACT AGG GAA GCA CAG ATG TGG AAA GTG AAT GTG CGG  96
Glu Lys Ala Ile Thr Arg Glu Ala Gln Met Trp Lys Val Asn Val Arg
            20                  25                  30

AAA ATG CCT TCA AAT CAG AAT GTT TCT CCA TCC CAG AGA GAT GAA GTA 144
Lys Met Pro Ser Asn Gln Asn Val Ser Pro Ser Gln Arg Asp Glu Val
        35                  40                  45

ATT CAA TGG CTG GCC AAA CTC AAG TAC CAA TTC AAC CTT TAC CCA GAA 192
Ile Gln Trp Leu Ala Lys Leu Lys Tyr Gln Phe Asn Leu Tyr Pro Glu
    50                  55                  60

ACA TTT GCT CTG GCT AGC AGT CTT TTG GAT AGG TTT TTA GCT ACC GTA 240
Thr Phe Ala Leu Ala Ser Ser Leu Leu Asp Arg Phe Leu Ala Thr Val
65                  70                  75                  80

AAG GCT CAT CCA AAA TAC TTG AGT TGT ATT GCA ATC AGC TGT TTT TTC 288
Lys Ala His Pro Lys Tyr Leu Ser Cys Ile Ala Ile Ser Cys Phe Phe
                85                  90                  95

CTA GCT GCC AAG ACT GTT GAG GAA GAT GAG AGA ATT CCA GTA CTA AAG 336
Leu Ala Ala Lys Thr Val Glu Glu Asp Glu Arg Ile Pro Val Leu Lys
            100                 105                 110

GTA TTG GCA AGA GAC AGT TTC TGT GGA TGT TCC TCA TCT GAA ATT TTG 384
Val Leu Ala Arg Asp Ser Phe Cys Gly Cys Ser Ser Ser Glu Ile Leu
        115                 120                 125

AGA ATG GAG AGA ATT ATT CTG GAT AAG TTG AAT TGG GAT CTT CAC ACA 432
Arg Met Glu Arg Ile Ile Leu Asp Lys Leu Asn Trp Asp Leu His Thr
    130                 135                 140

GCC ACA CCA TTG GAT TTT CTT CAT ATT TTC CAT GCC ATT GCA GTG TCA 480
Ala Thr Pro Leu Asp Phe Leu His Ile Phe His Ala Ile Ala Val Ser
145                 150                 155                 160

ACT AGG CCT CAG TTA CTT TTC AGT TTG CCC AAA TTG AGC CCA TCT CAA 528
Thr Arg Pro Gln Leu Leu Phe Ser Leu Pro Lys Leu Ser Pro Ser Gln
                165                 170                 175

CAT TTG GCA GTC CTT ACC AAG CAA CTA CTT CAC TGT ATG GCC TGC AAC 576
His Leu Ala Val Leu Thr Lys Gln Leu Leu His Cys Met Ala Cys Asn
            180                 185                 190
```

Fig. 2

```
CAA CTT CTG CAA TTC AGA GGA TCC ATG CTT GCT CTG GCC ATG GTT AGT  624
Gln Leu Leu Gln Phe Arg Gly Ser Met Leu Ala Leu Ala Met Val Ser
        195                 200                 205

CTG GAA ATG GAG AAA CTC ATT CCT GAT TGG CTT TCT CTT ACA ATT GAA  672
Leu Glu Met Glu Lys Leu Ile Pro Asp Trp Leu Ser Leu Thr Ile Glu
    210                 215                 220

CTG CTT CAG AAA GCA CAG ATG GAT AGC TCC CAG TTG ATC CAT TGT CGG  720
Leu Leu Gln Lys Ala Gln Met Asp Ser Ser Gln Leu Ile His Cys Arg
225                 230                 235                 240

GAG CTT GTG GCA CAT CAC CTT TCT ACT CTG CAG TCT TCC CTG CCT CTG  768
Glu Leu Val Ala His His Leu Ser Thr Leu Gln Ser Ser Leu Pro Leu
                245                 250                 255

AAT TCC GTT TAT GTC TAC CGT CCC CTC AAG CAC ACC CTG GTG ACC TGT  816
Asn Ser Val Tyr Val Tyr Arg Pro Leu Lys His Thr Leu Val Thr Cys
            260                 265                 270

GAC AAA GGA GTG TTC AGA TTA CAT CCC TCC TCT GTC CCA GGC CCA GAC  864
Asp Lys Gly Val Phe Arg Leu His Pro Ser Ser Val Pro Gly Pro Asp
        275                 280                 285

TTC TCC AAG GAC AAC AGC AAG CCA GAA GTG CCA GTC AGA GGT ACA GCA  912
Phe Ser Lys Asp Asn Ser Lys Pro Glu Val Pro Val Arg Gly Thr Ala
    290                 295                 300

GCC TTT TAC CAT CAT CTC CCA GCT GCC AGT GGG TGC AAG CAG ACC TCT  960
Ala Phe Tyr His His Leu Pro Ala Ala Ser Gly Cys Lys Gln Thr Ser
305                 310                 315                 320

ACT AAA CGC AAA GTA GAG GAA ATG GAA GTG GAT GAC TTC TAT GAT GGA 1008
Thr Lys Arg Lys Val Glu Glu Met Glu Val Asp Asp Phe Tyr Asp Gly
                325                 330                 335

ATC AAA CGG CTC TAT AAT GAA GAT AAT GTC TCA GAA AAT GTG GGT TCT 1056
Ile Lys Arg Leu Tyr Asn Glu Asp Asn Val Ser Glu Asn Val Gly Ser
            340                 345                 350

GTG TGT GGC ACT GAT TTA TCA AGA CAA GAG GGA CAT GCT TCC CCT TGT 1104
Val Cys Gly Thr Asp Leu Ser Arg Gln Glu Gly His Ala Ser Pro Cys
        355                 360                 365

CCA CCT TTG CAG CCT GTT TCT GTC ATG TAGTTTCAAC AAGTGCTACC TTTGAGT 1158
Pro Pro Leu Gln Pro Val Ser Val Met
    370                 375     377

GTAAACTAAG GTAGACTACT TTGGGAATGA GAACATCCAA AATCAGGAAA GGCTGTAGAA 1218

GGAAATATAC CTTAACAGGC TGATTTGGAG TGACCCAGAA AA                    1260
```

Fig. 2 (continued)

```
GGCACGAGGT TGTAGTTAAG CTCGTGTAAC GGCGGCGGTG TCGGTAGCTG CTGTAGCGAA 60

GAGAGTTTGG CGCG ATG TCT CAC ACC ATT TTG CTG GTA CAG CCT ACC AAG  110
                Met Ser His Thr Ile Leu Leu Val Gln Pro Thr Lys
                 1               5                  10

AGG CCA GAA GGC AGA ACT TAT GCT GAC TAC GAA TCT GTG AAT GAA TGC  158
Arg Pro Glu Gly Arg Thr Tyr Ala Asp Tyr Glu Ser Val Asn Glu Cys
        15                  20                  25

ATG GAA GGT GTT TGT AAA ATG TAT GAA GAA CAT CTG AAA AGA ATG AAT  206
Met Glu Gly Val Cys Lys Met Tyr Glu Glu His Leu Lys Arg Met Asn
    30                  35                  40

CCC AAC AGT CCC TCT ATC ACA TAT GAC ATC AGT CAG TTG TTT GAT TTC  254
Pro Asn Ser Pro Ser Ile Thr Tyr Asp Ile Ser Gln Leu Phe Asp Phe
45                  50                  55                  60

ATC GAT GAT CTG GCA GAC CTC AGC TGC CTG GTT TAC CGA GCT GAT ACC  302
Ile Asp Asp Leu Ala Asp Leu Ser Cys Leu Val Tyr Arg Ala Asp Thr
                65                  70                  75

CAG ACA TAC CAG CCT TAT AAC AAA GAC TGG ATT AAA GAG AAG ATC TAC  350
Gln Thr Tyr Gln Pro Tyr Asn Lys Asp Trp Ile Lys Glu Lys Ile Tyr
            80                  85                  90

GTG CTC CTT CGT CGG CAG GCC CAA CAG GCT GGG AAA TAATTGTGTT GGAAGC 402
Val Leu Leu Arg Arg Gln Ala Gln Gln Ala Gly Lys
        95                  100                 104

ACTGGGGGGG TTGGGGTGGG CTTGGAACAC AGGTGTGTAC AGCGTGCTGT AGTGGAAGTT 462
TTGTATCATA GTAATCCTGT TTCCACTTTG TTATACTCTA GCCAAGATTG ACTGTATTAG 522
ATGAAATGTG AGGATCTTGT TCAATCGGAA ACCCCCGTTA CCTCCTCTTT TTCTTTCTCT 582
TTCTTTTTTT TTTTTTACTT AAACATTTTT ATGATGATTT AGATGGAAGT TGTTCTTCGT 642
CACTTAATGT TGGTTCCAGT CCTTCAACTG TTCATATCTA CTTTATAACA TTCACATACT 702
AACCCTTCTG GGGTTCAAGA TGGGGGGTGG CAAATGCAGT TTAGCCATGT CCTCAAGATA 762
AAGTCTTGGT AAAAATAAAT AAATGTCCTT TAGTT                           797
```

Fig. 3

```
                  (A)
   1 GGAAGATGGC GGCTGTGGTG GAGAATGTAG TGAAGCTCCT TGGGGAGCAG TACTACAAAG
  61 ATGCCATGGA GCAGTGCCAC AATTACAATG CTCGCCTCTG TGCTGAGCGC AGCGTGCGCC
 121 TGCCTTTCTT GGACTCACAG ACCGGAGTAG CCCAGAGCAA TTGTTACATC TGGATGGAAA
 181 AGCGACACCG GGGTCCAGGA TTGGCCTCCG GACAGCTGTA CTCCTACCCT GCCCGGCGCT
 241 GGCGGAAAAA GCGGCGAGCC CATCCCCCTG AGGATCCACG ACTTTCCTTC CCATCTATTA
 301 AGCCAGACAC AGACCAGACC CTGAAGAAGG AGGGGCTGAT CTCTCAGGAT GGCAGTAGTT
 361 TAGAGGCTCT GTTGCGCACT GACCCCCTGG AGAAGCGAGG TGCCCCGGAT CCCCGAGTTG
 421 ATGATGACAG CCTGGGCGAG TTTCCTGTGA CCAACAGTCG AGCGCGAAAG CGGATCCTAG
 481 AACCAGATGA CTTCCTGGAT GACCTCGATG ATGAAGACTA TGAAGAAGAT ACTCCCAAGC
                                    (B)
 541 GTCGGGGAAA GGGGAAATCC AAGGGTAAGG GTGTGGGCAG TGCCCGTAAG AAGCTGGATG
 601 CTTCCATCCT GGAGGACCGG GATAAGCCCT ATGCCTGTGA CATTTGTGGA AAACGTTACA
 661 AGAACCGACC AGGCCTCAGT TACCACTATG CCCACTCCCA CTTGGCTGAG GAGGAGGGCG
 721 AGGACAAGGA AGACTCTCAA CCACCCACTC CTGTTTCCCA GAGGTCTGAG GAGCAGAAAT
 781 CCAAAAAGGG TCCTGATGGA TTGGCCTTGC CCAACAACTA CTGTGACTTC TGCCTGGGGG
 841 ACTCAAAGAT TAACAAGAAG ACGGGACAAC CCGAGGAGCT GGTGTCTTGT TCTGACTGTG
 901 GCCGCTCAGG GCATCCATCT TGCCTCCAAT TTACCCCCGT GATGATGGCG GCAGTGAAGA
 961 CATACCGCTG GCAGTGCATC GAGTGCAAAT GTTGCAATAT CTGCGGCACC TCCGAGAATG
1021 ACGACCAGTT GCTCTTCTGT GATGACTGCG ATCGTGGCTA CCACATGTAC TGTCTCACCC
1081 CGTCCATGTC TGAGCCCCCT GAAGGAAGTT GGAGCTGCCA CCTGTGTCTG GACCTGTTGA
                                    (C)
1141 AAGAGAAAGC TTCCATCTAC CAGAACCAGA ACTCCTCTTG ATGTGGCCAC CCACCTGCTC
1201 CCCGACATAT CTAAGGCTGT TTCTCTCCTC CACTTCATAT TTCATACCCA TCTTTCCCTT
```

Fig. 4

1261 CTTCCTCCTC TCCTTCACAA ATCCAGAGAA CCTTGGGGTG GTTGTGCCAG CCTGCCTTTG

1321 GCAGCTGCAA GCTGAGGTGG CAGCTCTGAC CACCTCTGGC CCCAGGCCTC AGGGAGAAAG

1381 GAGCAACACA CTGCCCCTAG GCGTGCGTGT GGCCCAGTTT CTCTCTGCTC TCCATTAAGT

1441 GCATTCACTC TGCTTGCCTT GGGCCCAGCC CCTGGTGATC ACAGGGTTCA AACAGTGTCC

1501 TCCTAGAAAG AGTGGGAGAG CAGCTCACTT CTCTGTGTTC TGCCTCCCCT CTGGTCTCCA

1561 GAGTTTTCCT GTCCTCTAGA GGCAAGCCAG GCCAGGGAGC TGGGAGCGAG CAAGCTGAGG

1621 CCACGTCCAC AAGGAGCTTT TCATGCCCCT GTGCCGCATA GCCTCACCTC TTTCCTCCAG

1681 AGTGGCTCTC TGCGGCCCTG TGTTCCTGCT ACAGAGTGTT CTTTTCTGGA GTCAGGATGT

1741 TCTCGGTCAC CCTCCTGGTT CTGCCCTGTC CCATTCCACC CCACCCCAGG GGGAACAGTA

1801 GCTTCACCTT GTTATTCCCA TTGCTCTCCT GGCTCACTCT TACGGTCGGT CTCCAGTGAC

1861 TGAAGCATTC CCCACCCTTG GAATTTCTCA TCTTCTGCCT CCCTTCCTAC TCCTTTTGGT

1921 TTTGTGGGGA GAGGGGAAGG ATCAGGGGGC AAGGCCAGCA GCTCGGGGGC CACAAGGAGA

1981 TGGATAATGT GCCTGTTTTT TAACACAACA AAAAAGCCTA CCTCCAAAAT CCCCTTTTTG

2041 TTCTTCCTGG ACCTGGGCAT TCAGCCTCCT GCTCTTAACT GAATTGGGAG CCTCTGCCAC

2101 CTGCCCCGTG TATCCTGGCT CTCAGCTCAT GGGGAAGCCA CATAGACATC CCTTTCTTCC

2161 CTTGCACGCT CGCTAGCAGC TGGTAGGTCT TCACACCCTG ATTCCTCAAG TTTTCTGCTT

2221 AGTGGCACTG ACATTAAGTA GTGGGGGGAC AGTCCATGCC AGGACACCCT GGAGTAGCCT

2281 TCCCCCTTGG CCGTGGGCAG GCCCTAACTC ACTGTCGCTT TGGAGTTGAG GTGTCTTTTT

2341 TTTTCTTTC TTTAGTTCCT GTATTCTAAA CATTAGTAAA AATAAATGTT TTTACACAG 2400

Fig. 4 (continued)

```
  1 ATG GCG GCT GTG GTG GAG AAT GTA GTG AAG CTC CTT GGG GAG CAG
    Met Ala Ala Val Val Glu Gln Val Val Lys Leu Leu Gly Glu Gln
    1               5                  10                  15

 46 TAC TAC AAA GAT GCC ATG GAG CAG TGC CAC AAT TAC AAT GCT CGC
    Tyr Tyr Lys Asp Ala Met Glu Gln Cys His Asn Tyr Asn Ala Arg
                20                  25                  30

 91 CTC TGT GCT GAG CGC AGC GTG CGC CTG CCT TTC TTG GAC TCA CAG
    Leu Cys Ala Glu Arg Ser Val Arg Leu Pro Phe Leu Asp Ser Gln
                35                  40                  45

136 ACC GGA GTA GCC CAG AGC AAT TGT TAC ATC TGG ATG GAA AAG CGA
    Thr Gly Val Ala Gln Ser Asn Cys Tyr Ile Trp Met Glu Lys Arg
                50                  55                  60

181 CAC CGG GGT CCA GGA TTG GCC TCC GGA CAG CTG TAC TCC TAC CCT
    His Arg Gly Pro Gly Leu Ala Ser Gly Gln Leu Tyr Ser Tyr Pro
                65                  70                  75

226 GCC CGG CGC TGG CGG AAA AAG CGG CGA GCC CAT CCC CCT GAG GAT
    Ala Arg Arg Trp Arg Lys Lys Arg Arg Ala His Pro Pro Glu Asp
                80                  85                  90

271 CCA CGA CTT TCC TTC CCA TCT ATT AAG CCA GAC ACA GAC CAG ACC
    Pro Arg Leu Ser Phe Pro Ser Ile Lys Pro Asp Thr Asp Gln Thr
                95                  100                 105

316 CTG AAG AAG GAG GGG CTG ATC TCT CAG GAT GGC AGT AGT TTA GAG
    Leu Lys Lys Glu Gly Leu Ile Ser Gln Asp Gly Ser Ser Leu Glu
                110                 115                 120
```

Fig. 5

361 GCT CTG TTG CGC ACT GAC CCC CTG GAG AAG CGA GGT GCC CCG GAT
Ala Leu Leu Arg Thr Asp Pro Leu Glu Lys Arg Gly Ala Pro Asp
125 130 135

406 CCC CGA GTT GAT GAT GAC AGC CTG GGC GAG TTT CCT GTG ACC AAC
Pro Arg Val Asp Asp Asp Ser Leu Gly Glu Phe Pro Val Thr Asn
140 145 150

451 AGT CGA GCG CGA AAG CGG ATC CTA GAA CCA GAT GAC TTC CTG GAT
Ser Arg Ala Arg Lys Arg Ile Leu Glu Pro Asp Asp Phe Leu Asp
155 160 165

496 GAC CTC GAT GAT GAA GAC TAT GAA GAA GAT ACT CCC AAG CGT CGG
Asp Leu Asp Asp Glu Asp Tyr Glu Glu Asp Thr Pro Lys Arg Arg
170 175 180

↓ (A)

541 GGA AAG GGG AAA TCC AAG GAG GCA AGC CAG GCC AGG GAG CTG GGA
Gly Lys Gly Lys Ser Lys Glu Ala Ser Gln Ala Arg Glu Leu Gly
185 190 195

586 GCG AGC AAG CTG AGG CCA CGT CCA CAA GGA GCT TTT CAT GCC CCT
Ala Ser Lys Leu Arg Pro Arg Pro Gln Gly Ala Phe His Ala Pro
200 205 210

631 GTG CCG CAT AGC CTC ACC TCT TTC CTC CAG AGT GGC TCT CTG CGG
Val Pro His Ser Leu Thr Ser Phe Leu Gln Ser Gly Ser Leu Arg
215 220 225

Fig. 5 (continued)

```
676 CCC TGT GTT CCT GCT ACA GAG TGT TCT TTT CTG GAG TCA GGA TGT
    Pro Cys Val Pro Ala Thr Glu Cys Ser Phe Leu Glu Ser Gly Cys
                    230                 235                 240

721 TCT CGG TCA CCC TCC TGG TTC TGC CCT GTC CCA TTC CAC CCC ACC
    Ser Arg Ser Pro Ser Trp Phe Cys Pro Val Pro Phe His Pro Thr
                    245                 250                 255
        ⬇Ⓑ                                     ⬇Ⓒ
766 CCA GGG GGA ACA GTA GCT TCA CCT TGT TAT TCC CAT TGC TCT CCT
    Pro Gly Gly Thr Val Ala Ser Pro Cys Tyr Ser His Cys Ser Pro
                    260                 265                 270

811 GGC TCA CTC TTA CGG TCG GTC TCC AGT GAC TGA 843
    Gly Ser Leu Leu Arg Ser Val Ser Ser Asp *
                    275                 280
```

Fig. 5 (continued)

```
  1 ATG GCG GCT GTG GTG GAG AAT GTA GTG AAG CTC CTT GGG GAG CAG
    Met Ala Ala Val Val Glu Asn Val Val Lys Leu Leu Gly Glu Gln
    1               5                  10                  15

 46 TAC TAC AAA GAT GCC ATG GAG CAG TGC CAC AAT TAC AAT GCT CGC
    Tyr Tyr Lys Asp Ala Met Glu Gln Cys His Asn Tyr Asn Ala Arg
                20                  25                  30

 91 CTC TGT GCT GAG CGC AGC GTG CGC CTG CCT TTC TTG GAC TCA CAG
    Leu Cys Ala Glu Arg Ser Val Arg Leu Pro Phe Leu Asp Ser Gln
                35                  40                  45

136 ACC GGA GTA GCC CAG AGC AAT TGT TAC ATC TGG ATG GAA AAG CGA
    Thr Gly Val Ala Gln Ser ASN Cys Tyr Ile Trp Met Glu Lys Arg
                50                  55                  60

181 CAC CGG GGT CCA GGA TTG GCC TCC GGA CAG CTG TAC TCC TAC CCT
    His Arg Gly Pro Gly Leu Ala Ser Gly Gln Leu Tyr Ser Tyr Pro
                65                  70                  75

226 GCC CGG CGC TGG CGG AAA AAG CGG CGA GCC CAT CCC CCT GAG GAT
    Ala Arg Arg Trp Arg Lys Lys Arg Arg Ala His Pro Pro Glu Asp
                80                  85                  90

271 CCA CGA CTT TCC TTC CCA TCT ATT AAG CCA GAC ACA GAC CAG ACC
    Pro Arg Leu Ser Phe Pro Ser Ile Lys Pro Asp Thr Asp Gln Thr
                95                  100                 105

316 CTG AAG AAG GAG GGG CTG ATC TCT CAG GAT GGC AGT AGT TTA GAG
    Leu Lys Lys Glu Gly Leu Ile Ser Gln Asp Gly Ser Ser Leu Glu
                110                 115                 120
```

Fig. 6

```
361 GCT CTG TTG CGC ACT GAC CCC CTG GAG AAG CGA GGT GCC CCG GAT
    Ala Leu Leu Arg Thr Asp Pro Leu Glu Lys Arg Gly Ala Pro Asp
                    125                 130                 135

406 CCC CGA GTT GAT GAT GAC AGC CTG GGC GAG TTT CCT GTG ACC AAC
    Pro Arg Val Asp Asp Asp Ser Leu Gly Glu Phe Pro Val Thr Asn
                    140                 145                 150

451 AGT CGA GCG CGA AAG CGG ATC CTA GAA CCA GAT GAC TTC CTG GAT
    Ser Arg Ala Arg Lys Arg Ile Glu Leu Pro Asp Asp Phe Leu Asp
                    155                 160                 165

496 GAC CTC GAT GAT GAA GAC TAT GAA GAA GAT ACT CCC AAG CGT CGG
    Asp Leu Asp Asp Glu Asp Tyr Glu Glu Asp Thr Pro Lys Arg Arg
                    170                 175                 180
                                    (A)
                                     ↓
541 GGA AAG GGG AAA TCC AAG GGG GGA ACA GTA GCT TCA CCT TGT TAT
    Gly Lys Gly Lys Ser Lys Gly Gly Thr Val Ala Ser Pro Cys Tyr
                    185                 190                 195
          (B)
           ↓
586 TCC CAT TGC TCT CCT GGC TCA CTC TTA CGG TCG GTC TCC AGT GAC
    Ser His Cys Ser Pro Gly Ser Leu Leu Arg Ser Val Ser Ser Asp
                    200                 205                 210

631 TGA 633

    *
```

Fig. 6 (continued)

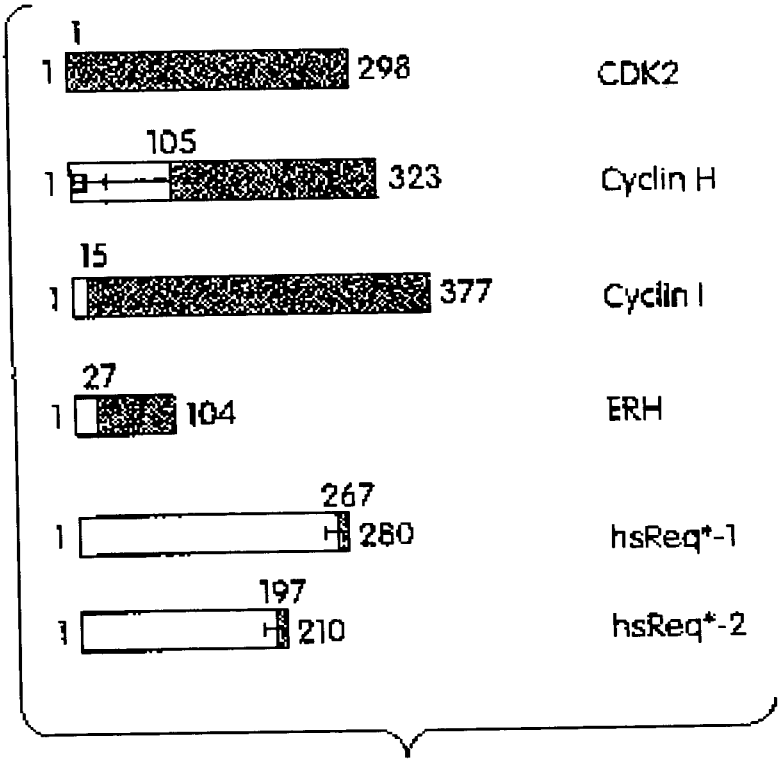
1
1
298
CDK2
105
1
323
Cyclin H
15
1
377
Cyclin I
27
1
104
ERH
267
1
280
hsReq*-1
197
1
210
hsReq*-2

Fig. 7

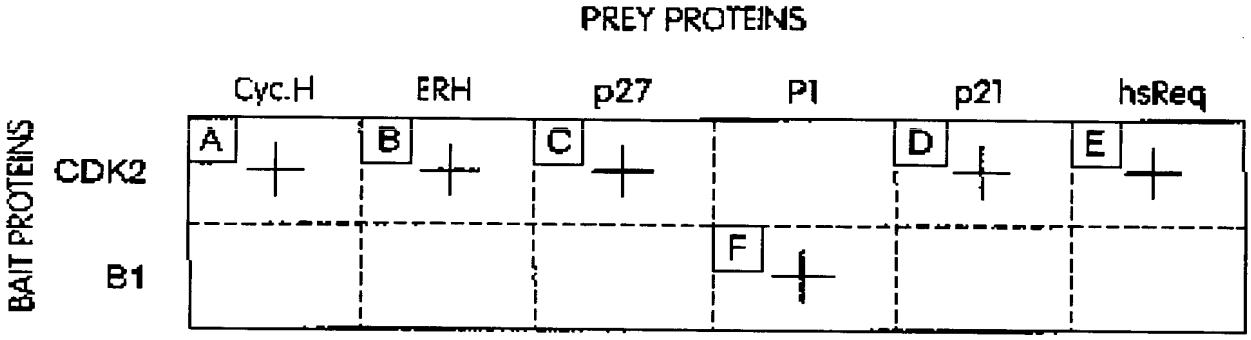

Fig. 8

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 15/62, 1/19, C07K 14/47, C12Q 1/00, 1/68, G01N 33/68, 33/566, A01K 67/027

A3

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(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/969,106 (CIP)
Filed on 13 November 1997 (13.11.97)

(71) Applicant *(for all designated States except US)*: CURAGEN CORPORATION [US/US]; 11th floor, 555 Long Wharf Drive, New Haven, CT 06511 (US).

(72) Inventors; and
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(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky, and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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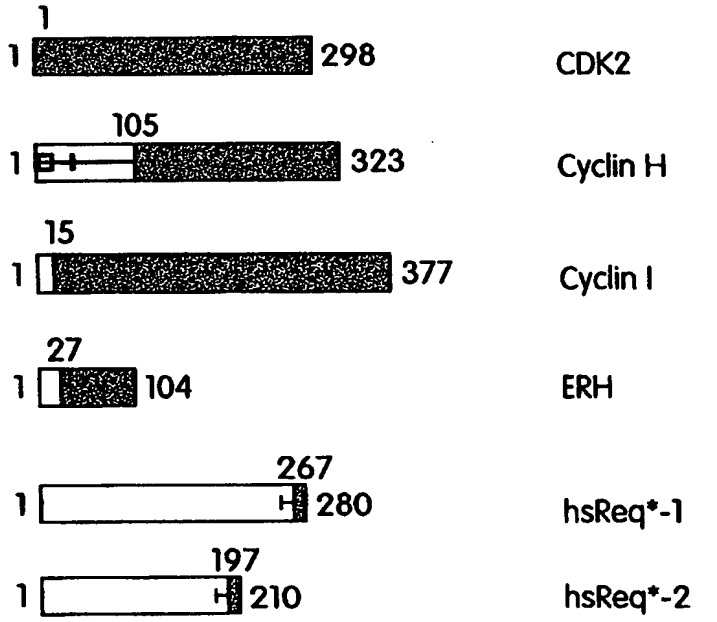
10 September 1999 (10.09.99)

(54) Title: CDK2 PROTEIN AND CDK2 PROTEIN COMPLEXES

(57) Abstract

The present invention discloses complexes of the CDK2 protein with proteins identified as interacting with the CDK2 protein (CDK2 protein–IPs) by a modified, improved yeast two hybrid assay system. The proteins which were identified to interact with the CDK2 protein, and thus form complexes, included: cyclin I, ERH, hsReq*–1 and hsReq*–2, as well as derivatives, fragments analogs and homologs thereof. The invention also provides nucleic acids encoding the hsReq*–1 and hsReq*–2 nucleotide sequences, and proteins and derivatives, fragments and analogs thereof. Methodologies of screening these aforementioned complexes for efficacy in treating and/or preventing various diseases and disorders, particularly neoplasia and atherosclerosis, are also disclosed herein.



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INTERNATIONAL SEARCH REPORT

International Application No
PC./US 98/24095

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C12N1/19 C07K14/47 C12Q1/00
C12Q1/68 G01N33/68 G01N33/566 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 11176 A (COLD SPRING HARBOR LAB ;ZHANG HUI (US); BEACH DAVID (US)) 27 March 1997 (1997-03-27) abstract page 2, line 34 - page 3, line 2 page 48, line 30 - line 37 --- -/--	5-7,13, 16,35, 36,40,65 1-4, 8-12,14, 15, 32-34, 37-39, 41,42, 47,49, 58-64, 67-78

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
31 March 1999	23.07.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Jansen, K-S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 25345 A (LILLY CO ELI ;KOVACEVIC STEVEN (US); OTTO KEITH A (US); RAO RAMACH) 17 July 1997 (1997-07-17) abstract; claim 1; examples	5-7,13, 16,35, 36,40,65
X	WO 97 12973 A (SUMITOMO ELECTRIC INDUSTRIES) 10 April 1997 (1997-04-10)	8,51,78
A	abstract; claims; examples	1-7, 9-16, 32-42, 47,49, 52-54, 58-65, 67-77
	-& EP 0 863 204 A (SUMITOMO ELECTRIC INDUSTRIES) 9 September 1998 (1998-09-09)	
X	WADE HARPER ET AL: "The p21 Cdk-Interacting Protein Cip1 Is a Potent Inhibitor of G1 Cyclin-Dependent Kinases" CELL, vol. 75, 19 November 1993 (1993-11-19), pages 805-816, XP002098279	8,51
A	see abstract; introduction; discussion	1-7, 9-16, 32-42, 47,49, 52-54, 58-65, 67-78
	page 808, right-hand column, line 2 - line 7	
A	WO 97 27297 A (MITOTIX INC) 31 July 1997 (1997-07-31)	49, 51-54, 58-65, 67-78
	page 1, line 5 - page 2, line 34 page 19, line 12 - page 20, line 8	

6

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US 98/24095

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 49,52-54,58-62,68-70,75-77 (all partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

See extra sheet, Invention 1.

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The modulating molecules of claims 52-54,58-62,69-72,76-77 have not been sufficiently characterised according to Article 6 PCT, thereby precluding a meaningful and complete search.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: (1-16,32-42,47,49,51-54,58-65,67-78) - partial

A purified complex of CDK2 protein and cyclin I.

A chimaeric protein comprising a fragment of CDK2 and a fragment of cyclin I.

Corresponding antibodies, nucleic acids, vectors and recombinant cells, pharmaceutical compositions; uses in diagnosis and treatment of disease; methods of screening for ligands and modulators and their use; kits.

Transgenic animals (including knock-outs).

2. Claims: (1-16,32-42,47,49,51-54,58-65,67-78) - partial

Idem as subject matter 1, wherein the protein forming a complex with CDK2 is ERH.

3. Claims: (17-31,43-46,48,50,55-57,66) - complete; (1-16, 32-42,47,49,51-54,58-65,67-78) - partial

Idem as subject matter 1, wherein the protein forming a complex with CDK2 is hsReq*-1 or hsReq*-2.

Corresponding antibodies, nucleic acids, vectors and recombinant cells, pharmaceutical compositions; uses in diagnosis and treatment of disease; methods of screening for ligands and modulators and their use also claimed alone for hsReq*-1 and hsReq*-2 (SEQ IDs 8-13).

4. Claims: (5,6,7,13,16,35,36,40,65,71) - partial

A chimaeric protein comprising a fragment of a CDK2 protein consisting of at least 6 amino acids fused, via a covalent bond, to a fragment of a CDK2-interacting protein (insofar as not covered by inventions 1-3) consisting of at least 6 amino acids.

5. Claims: (75) - partial

A method of monitoring the efficacy of treatment of a disease or disorder characterised by an aberrant level of the CDK2 protein and a CDK2-interacting protein (insofar as not covered by inventions 1-3) in a subject.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna... Application No
PCT/US 98/24095

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9711176 A	27-03-1997	CA 2230138 A	27-03-1997
WO 9725345 A	17-07-1997	AU 1526897 A	01-08-1997
		CA 2241849 A	17-07-1997
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		AU 700847 B	14-01-1999
		AU 1747397 A	20-08-1997
		CA 2242960 A	31-07-1997
		EP 0877804 A	18-11-1998